UNIVERSITY OF BRISTOL

THE ANNUAL REPORT

OF THE

Agricultural and Horticultural Research Station

(THE NATIONAL FRUIT AND CIDER INSTITUTE)

LONG ASHTON, BRISTOL

1955

BATH :
PRINTED BY THE MENDIP PRESS LTD.

AGRICULTURAL COMMITTEE, 1955-56.

Appointed by the Ministry of Agriculture, Fisheries and Food.

A. WOODALL, Esq., 34, Weoley Hill, Selly Oak, Birmingham 29.

Appointed by the National Fruit and Cider Institute.

CAPTAIN D. M. WILLS, C.B.E. (*Chairman*), Barley Wood, Wrington, Somerset.
C. P. NORBURY, Esq., Sherridge, near Malvern, Worcestershire.
T. N. HOBLYN, Esq., East Malling Research Station, near Maidstone, Kent.
E. BALL, Esq., Oldfield, Pipe and Lyde, Hereford.
GILES TUKER, Esq., Butts Green Orchards, Danbury, Essex.
M. B. CRANE, Esq., F.R.S., Firs Farm, Haughley, near Stowmarket, Suffolk.
T. W. BARNES, Esq., Showle Court, Yarkhill, Ledbury, Herefordshire.

Appointed by the Senate of the University of Bristol.

PROFESSOR T. WALLACE, C.B.E., M.C., D.Sc., V.M.H., F.R.S., Professor of Horticultural Chemistry in the University.
PROFESSOR WILSON BAKER, M.A., D.Sc., F.R.S., Professor of Organic Chemistry in the University.
PROFESSOR J. E. HARRIS, M.A., Ph.D., Professor of Zoology in the University.
PROFESSOR A. MESSERVY, M.R.C.V.S., Professor of Veterinary Surgery in the University.

Appointed by the Council of the University of Bristol.

THE VICE-CHANCELLOR (SIR PHILIP MORRIS, C.B.E., M.A., LL.D.), The University, Bristol.
ANDREW HAGGARD, Esq., The Hilltop Fruit Farm, Ledbury, Herefordshire.
SIR ARTHUR HOBHOUSE, M.A., J.P., Hadspen House, Castle Cary, Somerset.
THE RIGHT HON. EARL WALDEGRAVE, Chewton House, Chewton Mendip, Bath.
PROFESSOR J. L. M. MORRISON, D.Sc., Professor of Mechanical Engineering in the University.

Appointed by the Gloucestershire County Council.

H. R. STEPHENS, Esq., M.B.E., Hortham Farm, Almondsbury, Nr. Bristol.

Appointed by the Somerset County Council.

MAJOR W. F. Q. SHULDHAM, East Stoke House, Stoke-under-Ham, Somerset.

Appointed by the Wiltshire County Council.

O. M. PEALL, Esq., Hatfield Farm, Oare, Marlborough, Wiltshire.

Appointed by the Food Investigation Board.

R. G. TOMKINS, Esq., M.A., Ph.D., Ditton Laboratory, Larkfield, Maidstone, Kent.

Appointed by the National Association of Cider Makers.

N. W. BEVERIDGE, Esq., Taunton Cider Company, Norton Fitzwarren, Somerset.

Appointed by the Apple Juice Producers' Association of Great Britain.

V. L. S. CHARLEY, Esq., Ph.D., 40, Boxbush Road, Coleford, Gloucestershire.

Co-opted Members.

A. ESMOND ROBINSON, Esq., O.B.E., M.C., The Batch, Flax Bourton, near Bristol.
C. E. HUDSON, Esq., C.B.E., Senior Education and Advisory Officer, Ministry of Agriculture, Fisheries and Food, Great Westminster House, Horseferry Road, London, S.W.1.

Secretary.

THE REGISTRAR AND SECRETARY OF THE UNIVERSITY,
H. C. BUTTERFIELD, M.A.

LIST OF STAFF—1955.

Long Ashton Research Station.

Director :
PROFESSOR T. WALLACE, C.B.E., M.C., D.Sc., F.R.I.C., V.M.H., F.R.S.

Secretary :
A. L. LEGGETT, A.S.A.A.

Nutrition of Fruit Plants :
C. BOULD, M.Sc., Ph.D.
D. G. HILL-COTTINGHAM, M.Sc.
E. G. BRADFIELD, A.R.I.C.
G. V. C. DAVIES, B.Sc. (*Appointed* 4 *April*, 1955).
MRS. B. MERCHANT (née Hood). (*Resigned* 30 *November*, 1955).

Pomology and Plant Breeding :
*L. C. LUCKWILL, B.Sc., Ph.D.
E. W. HOBBIS, B.Sc., N.D.H. (Plantations Officer).
D. L. ABBOTT, B.Sc.
R. R. WILLIAMS, B.Sc., Dip. Hort. Sci.
D. WILSON, B.Sc. (Plant Breeder). (*Appointed* 1 *October*, 1955).
MISS B. A. RAKE, B.Sc., N.D.H.
P. S. WEAVER.
MISS A. J. BEAGLES.

Plant Physiology :
E. J. HEWITT, B.Sc., Ph.D., A.K.C. (*Seconded to A.R.C. Unit of Plant Nutrition*).

Chemistry of Insecticides and Fungicides :
J. T. MARTIN, B.Sc., Ph.D., D.Sc., F.R.I.C.
D. WOODCOCK, M.Sc., Ph.D., F.R.I.C. (Organic Chemistry).
W. D. E. THOMAS, B.Sc., Ph.D., A.R.I.C. (Physical Chemistry).
E. SOMERS, M.Sc.
G. V. COLES, B.Sc. (*Resigned* 31 *January*, 1955).
C. P. LLOYD-JONES, B.Sc., A.R.I.C.
E. J. SKERRETT, Ph.D., A.R.I.C. (*Appointed* 1 *May*, 1955).
R. F. BATT.
A. J. SIMS, A.R.I.C.
J. F. HARRIS, A.R.I.C.
J. A. PICKARD.
E. A. BAKER.
D. R. CLIFFORD. (*Appointed* 1 *January*, 1955).
MISS P. M. JACKSON.

Entomology :
H. G. H. KEARNS, O.B.E., B.Sc., Ph.D., Dip. Agric.
S. H. BENNETT, B.Sc., Ph.D.
N. G. MORGAN, B.Sc.
A. STRINGER, B.Sc., A.R.C.S.
C. G. L. FURMIDGE, B.Sc., Ph.D., A.R.I.C. (Physical Chemistry).
T. E. COBBALD, B.Sc., G.I.Mech.E. (*Appointed* 1 *August*, 1955).
E. A. FRIBBINS. (*Appointed* 1 *February*, 1955).
A. H. WILKINS.
MRS. E. B. SALWAY.
MISS M. P. SCADDING.
J. H. RUSSELL.

**Visiting Professor in Pomology, Cornell University, U.S.A., from* 1 *July*, 1954, *to* 1 *August*, 1955.

LIST OF STAFF—*continued.*

Plant Pathology :
R. W. Marsh, M.A.
R. J. W. Byrde, B.Sc. (Hort.), Ph.D.
A. T. K. Corke, B.Sc., Ph.D.
A. H. Fielding. (*On National Service from* 26 *October*, 1954).
Miss N. M. Waugh.
Miss M. Marlow.

Cider and Fruit Juices :
A. Pollard, M.Sc., Ph.D.
Miss M. E. Kieser, B.Sc., A.R.I.C. (Chemistry).
L. F. Burroughs, B.Sc., A.R.I.C. (Chemistry).
F. W. Beech, B.Sc., A.R.I.C. (Microbiology).
C. G. Whiting, B.Sc., Ph.D. (Chemistry).
C. F. Timberlake, M.Sc., A.R.I.C. (Chemistry).
J. G. Carr, B.Sc. (Microbiology).
R. J. Hathway (Cider-maker).
J. H. Llewellyn.
Mrs. M. R. Rodgers.
P. E. W. Rodgers.
Miss M. A. S. Cousin, B.Sc.
R. A. Coggins.
B. L. Davies. (*Appointed* 21 *March*, 1955).
Mrs. J. M. Fielding.
G. Bradshaw.
D. J. Sissons.

Domestic Food Preservation :
Miss B. A. Crang, B.Sc.
Miss M. Sturdy, B.Sc. (H. & S.S.).
Miss S. J. Perkins, B.Sc. (*Resigned* 1 *July*, 1955).
Miss J. Sherborne, B.Sc. (*Resigned* 30 *November*, 1955).
Mrs. J. M. Bowcott, B.Sc. (*Appointed* 10 *October*, 1955).

Willows :
K. G. Stott, B.Sc.

Statistics :
G. M. Clarke, M.A., Dip. Stat.
Miss M. E. Holgate, M.A. (*Appointed* 1 *January*, 1955).

Scientific Liaison :
M. Greenwood, O.B.E., M.Sc. (Scientific Liaison Officer).
G. E. Clothier (Station Guide and Meteorological Recorder).

Library :
G. T. Spinks, M.A. (Librarian) (*part-time*).
Miss I. E. Taylor.

Laboratory Staff :
F. E. Maltby, B.E.M. (Chief Laboratory Steward).
G. Kitchingham.

Photography :
G. H. Jones, A.R.P.S.
E. R. Wilkins.

Plantations and Greenhouses :
E. W. Hobbis, B.Sc., N.D.H. (Plantations Officer).
S. H. Legge (Marketing Officer and Recorder).
E. Catlow (Recorder).
A. I. Campbell, N.D.H. (Propagator and Recorder).
R. M. Jarrett (Recorder).
C. W. Harper (Spraying Recorder).
D. W. Burd, N.D.H. (Recorder). (*Resigned* 31 *December*, 1955).
A. J. Hurdle (Plantations Foreman).
Mrs. R. Lewis (Assistant Recorder). (*Resigned* 31 *March*, 1955).
N. T. Weatheritt (Assistant Recorder).
(*Appointed* 1 *April*, 1955).
J. G. Clarke (Greenhouse Foreman).

LIST OF STAFF—*continued.*

Workshops and Maintenance :	S. E. Cole. H. J. Jones. B. F. Shearn.
Canteen :	Mrs. N. M. King.
Administrative and Clerical Staff :	R. A. Merchant (Chief Clerk, Accounts). D. H. Pittman (Cashier). Miss M. H. Simpson (Director's Secretary). J. Packer (Senior Clerk). Miss E. M. Matthews. Miss B. J. Haysham. Miss N. K. Bullock. T. J. Ford. Miss J. M. Walters. Mrs. M. Wippell. Miss D. M. Smith. Miss M. J. Hathway.
Outdoor and Cider House Staff with more than 20 years' service :	R. Jones (33). J. C. W. Barnes (27). W. M. Hynam (27). F. W. Hynam (26). K. J. Escott (25).

Agricultural Research Council Unit of Plant Nutrition (Micro-Nutrients).

Honorary Director :
Professor T. Wallace, C.B.E., M.C., D.Sc., F.R.I.C., V.M.H., F.R.S.

R. A. Webb, B.A., D.Phil. (*Appointed* 1 *June*, 1955).
E. J. Hewitt, B.Sc., Ph.D., A.K.C. (Seconded to Unit).
D. J. D. Nicholas, B.Sc., Ph.D., A.K.C., F.R.I.C.
W. Plant, B.Sc. (Hort.), Ph.D., N.D.H. (*Resigned* 31 *March*, 1955).
A. H. Williams, M.A., B.Sc.
H. M. Stevens, B.Sc., Ph.D.
A. J. Abbott, M.Sc., Dip. Hort.
W. J. Redmond.
Mrs. O. M. James, B.Sc. (Hort.). (*Resigned* 31 *January*, 1955).
D. J. Fisher, A.R.I.C.
B. A. Notton, A.R.I.C. (*On National Service from* 5 *October*, 1954).
D. G. Hallas.
H. M. Webber, B.Sc., A.R.I.C. (*Resigned* 27 *August*, 1955).
C. S. Gundry, S.D.H. (Greenhouse Experiments Officer).
Miss E. Atkinson, B.Sc.
J. Jarrett.
Miss T. C. N. Goodman, B.Sc. (*Appointed* 1 *October*, 1955).
J. H. Scawin, A.R.I.C. (*Appointed* 1 *October*, 1955).
D. P. Hucklesby, B.Sc. (*Appointed* 1 *December*, 1955).
M. A. Wright.
Miss R. A. Paget. (*Resigned* 13 *August*, 1955).
M. W. J. Heyes.

CONTENTS.

PAGE

Introduction .. .. *T. Wallace, Director* 9

Summary of Research, 1955 25

Pomology :—

Stembuilders for cider trees : nursery trials .. .. *A. I. Campbell* 46
Virus diseases of fruit trees : V. Experiments on rubbery wood, mosaic and flat limb of apples .. .. *L. C. Luckwill* 51
Growth substances as fruit-thinning agents for apples : progress report *D. L. Abbott* 58
A note on ten years' cropping of black currants at Long Ashton *E. W. Hobbis and E. Catlow* 61
Fruit drop in black currants : I. Factors affecting "running-off" *S. S. Teaotia and L. C. Luckwill* 64
Fruit drop in black currants : II. The effect of naphthalene acetic acid *L. C. Luckwill* 75
The propagation of gooseberries : III. The effect of time of taking and planting hardwood cuttings and the influence of buds on rooting .. *Barbara A. Rake* 78

Plant Nutrition : Fruit :—

The use of iron chelates for the control of lime-induced chlorosis in fruit trees *C. Bould* 87
Foliar analysis in relation to black currant nutrition : I. Investigations on leaf disc sampling .. .. *C. Bould and E. G. Bradfield* 93

Plant Pathology :—

Spray application problems : XXI. The rapid assessment of deposits of copper fungicides .. .. *J. T. Martin* 98
Spray application problems : XXII. Determination of captan deposits *J. T. Martin and J. A. Pickard* 103
Spray application problems : XXIII. Determination of deposits of DDT, *gamma*–BHC and chlorbenside .. .. .. *J. T. Martin and R. F. Batt* 106
The role of supplements in copper fungicide sprays .. .. .. *E. Somers* 111
Radioactive tracer studies during the period 1953–55 .. .. *C. P. Lloyd-Jones* 116
An unusual attack on apples by a sawfly (*Ametastegia* sp.) .. .. *S. H. Bennett* 120
Spray application problems : XXIV. The study of plant surfaces and spray deposits by means of transparent impressions *S. H. Bennett and C. G. L. Furmidge* 121
Spray application problems : XXV. The application of chlorbenside and chlorfensone for the control of the winter eggs of the Red Spider mite (*Metatetranychus ulmi*, Koch) *H. G. H. Kearns, N. G. Morgan and A. H. Wilkins* 125
Spray application problems : XXVI. The range of fungicidal action of micro-deposits of captan on artificial surfaces and on leaves .. .. *D. J. Butt* 127
Spray application problems : XXVII. Field experiments in the control of apple and pear scab by small-volume sprays, 1955 *H. G. H. Kearns, R. W. Marsh and N. G. Morgan* 136
Spraying experiments against apple scab at Long Ashton, 1955 *R. J. W. Byrde, G. M. Clarke and C. W. Harper* 140
Spray application problems : XXVIII. A compressed air spray nozzle for laboratory and field equipment for small-volume air-flow spraying *H. G. H. Kearns and N. G. Morgan* 146
Spray application problems : XXIX. A tractor-driven air-flow mist-blower for experimental work .. .. .. *H. G. H. Kearns and N. G. Morgan* 151
Spray application problems : XXX. Mobile observation platforms for the examination of tree crops .. .. *H. G. H. Kearns and N. G. Morgan* 156

Cider and Fruit Juices :—

Acid changes in cider fermentations : I. The significance of acid changes in cider-making .. .. *F. W. Beech and A. Pollard* 159
Acid changes in cider fermentations : II. The effect of juice composition *J. G. Carr and G. C. Whiting* 163
Acid changes in cider fermentations : III. The effect of sulphite treatments *L. F. Burroughs, F. W. Beech and G. C. Whiting* 169
Acid changes in cider fermentations : IV. Yeast autolysis and cider stability *L. F. Burroughs* 174

CONTENTS—*continued.*

PAGE

Domestic Food Preservation :—

A comparison of some varieties of vegetables preserved by canning and by freezing : progress report IV .. .. *Alice Crang and Margaret Sturdy* 178
A comparison of some varieties of fruit preserved by bottling and by freezing : progress report VI .. .. *Alice Crang and Margaret Sturdy* 182
Domestic freezing of pork and pork products .. *Margaret Sturdy and Alice Crang* 188

Statistics :—

A rapid method of assessing black currant leaf spot *G. M. Clarke and A. T. K. Corke* 196

Meteorology :—

Meteorological records, 1955 .. .. *G. E. Clothier* 201

A.R.C. Unit of Plant Nutrition (Micronutrients) :—

Factors affecting the activity of nitrate reductase in cauliflower plants *E. J. Hewitt, E. G. Fisher and M. I. Candela* 202
The destruction of ascorbic acid by tissue preparations of cauliflower grown with different nitrogen and molybdenum supplies .. .. .. *E. J. Hewitt* 211

Publications, 1955 221

Copyright of all articles and illustrations in this Report reserved by the University of Bristol Agricultural and Horticultural Research Station, Long Ashton.

INTRODUCTION.

By T. WALLACE, Director.

The year under review has been marked by steady progress in the major activities of the Institute.

Close collaboration with the N.A.A.S. has been maintained. The annual refresher course for N.A.A.S. Horticultural Officers and officers of County Council staffs was held at the Research Station on 12th and 13th July. In addition the N.A.A.S. Conference of Analysts, which met for its annual meeting in Bristol, visited the Institute on 11th May, when its members spent the day visiting the chemical laboratories to inspect apparatus and equipment and to discuss the latest analytical methods. A special two-day grafting course for N.A.A.S. horticultural officers, similar to that in 1954, was again held in March.

The Director has continued to keep in close touch with the activities of the N.A.A.S. through his membership of a number of official committees, including the Agricultural Improvement Council and its Horticultural Committee, of which he is chairman, its Fruit Working Party, and the Advisory Committees of the Horticultural Experimental Stations at Luddington, Efford, Rosemaund and Rosewarne. He has also continued to serve as Chairman of the N.A.A.S. Fruit Group which is responsible for the Ministry of Agriculture bulletins and leaflets on fruit culture.

The Station has also participated actively in a number of fruit experiments at the horticultural stations, and during the year undertook responsibility for a new experiment on apple manuring at the Efford Station.

The collaboration with the Colonial Office referred to in previous reports was further developed during the year by the appointment of two officers at Long Ashton under the scheme agreed between the Colonial Office and the Station through the Agricultural Research Council. Both the officers appointed will work directly under Dr. Kearns, one on problems of practical entomology and spraying at centres in the Colonial Territories, whilst the other will assist with engineering problems in the workshops at Long Ashton. The third officer envisaged in the scheme, also for work abroad, has not so far been appointed, but it is hoped that the vacancy will be filled early in 1956.

In connection with this scheme, Dr. Kearns made two visits abroad during 1955—in January to Ulm, Germany, to inspect apparatus for use in the examination of coconut palms in Zanzibar, and in November-December to Jamaica to inspect the results of spraying trials on Leaf Spot disease of bananas.

In addition to the work of Dr. Kearns, the Station has participated in other investigations in the Colonial Territories through Dr. R. A. Webb, who joined the Station staff (Unit of Plant Nutrition) from his service in The Gambia in June. Dr. Webb, although now stationed at Long Ashton, will continue to work on problems of crop production in The Gambia and during 1955 he has, in fact, directed a series of field experiments on fertilizer use, which have been planned as the result of his previous investigations in the Colony. Dr. Webb inspected the experiments during a visit paid in the October-November period, and is now awaiting the data on crop yields to

make a final assessment of the effects of the fertilizer treatments. As the Colonial Office is anxious that Dr. Webb's work in The Gambia shall be continued, arrangements have been made for him to train officers at Long Ashton and to continue the laboratory and field experiments begun by him.

In the 1954 Report reference was made to a visit made by Dr. Hewitt to the West African Institute for Oil Palm Research at Benin, Nigeria, to examine nutritional problems of the oil palm and to make proposals for the establishment of a unit for nutritional investigations on this crop. Following the submission of his report the Management Committee of the Institute have accepted Dr. Hewitt's recommendations and a Unit has already been established. In preparation for the working of the Unit, Mr. R. A. Bull, Pathologist at the Institute, spent the summer months of 1955 at Long Ashton learning the special pot culture methods in use at the Station. We are very glad to have been of service in the establishment of this new unit and we look forward to further collaboration in the nutritional problems of this important West African crop.

Following the completion of the work undertaken on behalf of the Rubber Research Institute of Malaya on the fungicidal constituents of tar oil washes, the final report has been forwarded to Malaya and a letter of appreciation received from the Director of the Rubber Research Institute, Malaya, recording his thanks and appreciation of the collaboration established between the two institutes in carrying out the investigations.

Both sub-committees of the Cider Advisory Committee met during the year. At the meeting of the Cider Orcharding Sub-Committee held on 28th March, the main item of business was to consider the report of the special sub-committee appointed to revise the 1947 list of cider varieties recommended for planting. This list has been subjected to some criticism after further experience of some of the varieties, and particularly because of the lack of sharp and bitter sharp varieties in the list. The meeting agreed to recommend a number of deletions from the 1947 list, but could not recommend the inclusion of further varieties. This latter point is to be studied further by the special sub-committee.

The Cider Manufacture Sub-Committee, which met on 28th October to consider the research programme of the Cider Section, resolved to extend the membership of the sub-committee by inviting some of the larger firms in the industry, not already represented, to nominate technical representatives. The National Association of Cider Makers have also been invited to make proposals for extending the membership.

A pamphlet on the cider industry has been prepared by Dr. Pollard for publication by the Bath and West and Southern Counties Society. The publication should be available early in 1956.

Members of the A.R.C. Cider and Fruit Juices Committee, under the chairmanship of Sir John Simonsen, visited the Station on 25th April to inspect the work and to discuss some of the research problems under investigation with the staff of the Cider and Fruit Juices Section. Visits of this nature have proved of great value to the staff and it is to be hoped that similar visits will be paid in future years. Sir John Simonsen also visited the Station on 20th October, when details of the research report and programme of work were discussed. We are grateful to Sir John and to the Committee for their continued help and interest.

During the year the Institute received as a gift a first edition copy, published in 1811, of " Pomona Herefordiensis " by Thomas Andrew Knight, from Mr. and Mrs. Arthur Amos of Spring Grove Farm, Wye, Kent. The generosity of Mr. and Mrs. Amos in presenting this rare and valued volume to the Station is much appreciated and we would wish here to record our best thanks.

Substantial progress has now been made in the research work on meat and poultry preservation, and this enabled three courses to be given in March and April, 1955. Those attending comprised selected County Instructresses in Rural Domestic Economy, Ministry of Education representatives, and lecturers from Domestic Science Training Colleges.

The annual meeting of the British Association for the Advancement of Science, held in Bristol in September, provided a special feature of the year. Members of the Station staff contributed papers in Sections K (Botany) and M (Agriculture), and exhibits were staged at the Institute for the benefit of visitors from the Association. In addition to casual visitors three organized parties were received and entertained as follows : Section K (Botany-Forestry) —125 members ; Section M (Agriculture)—70 members ; Section I (Physiology) —50 members. The Director performed the duties of Vice-President of Sections K and M and Dr. Martin of Secretary of Section M.

On July 21st we were honoured by a visit of two members of the Agricultural Research Council, Professor S. J. Watson, Vice-Chairman, and Mr. David Lowe. In addition to meeting members of staff and discussing research projects, Professor Watson and Mr. Lowe met the Chairman and members of the Finance and Business Sub-Committee at lunch and attended for a short while a meeting of the committee. We hope that this visit will be the forerunner of others by members of the Council.

In December we also received a visit from Professor and Mrs. E. L. Proebsting of the University of California Agricultural Experiment Station, Davis, California. Professor Proebsting spent a year working at Long Ashton in 1930, when he carried out a chemical investigation jointly with the present Director. It was particularly pleasing to the older members of staff to renew this friendship and it was a special pleasure to have our visitors present at our annual Christmas Party and lunch.

Dr. Elwood G. Fisher, Associate Professor of Pomology, Cornell University, New York, spent the period September 1955 to January 1956 working in our plant nutrition laboratory in collaboration with Dr. Hewitt. Dr. Fisher's stay with us has forged yet another link with the Pomology Department of Cornell, where Dr. Luckwill recently spent a year as Visiting Professor of Pomology, returning to Long Ashton in August.

A further pleasant feature of 1955 was the visit paid by the Director, Dr. J. Philp, and members of the staff of the National Vegetable Research Station, Wellesbourne, on 9th July. The visit comprised a short tour of the Station during the morning, followed by lunch in the ciderhouse and then cricket and tennis matches at the University Sports Ground, Coombe Dingle. At this first sports meeting the honours between the two stations were even : Wellesbourne defeated us at tennis and Long Ashton won the cricket match. The occasion was much enjoyed by both staffs and it is hoped the event, alternating between the two stations, will prove an annual " fixture."

During the year four members of the assistant staff, Messrs. J. F. Harris, J. H. Scawin, A. J. Sims and H. M. Webber, were successful in the A.R.I.C. examination of the Royal Institute of Chemistry ; in addition, Mr. Webber obtained the degree of B.Sc. (Special) of the University of London. These outstanding successes have been achieved as the result of attending the day and evening courses at the College of Technology, Bristol, under the release schemes for study of the Ministry of Agriculture and the University.

Hitherto the main courses provided by the College of Technology have been in Chemistry, but recently we have discussed with Dr. Bolland, the Head of the Chemistry and Biology Department of the College, the possibility of initiating courses in Biology similar to those in Chemistry, leading to National Certificates such as are now available in Chemistry. Dr. Bolland is hopeful that this can be done and we have assured him of our active co-operation in his endeavours.

Mr. R. O. Sharples, B.Sc., was awarded the first of the three Boots Scholarships available during the second period of the scheme. Mr. Sharples had previously held a postgraduate research studentship at the Royal Holloway College, London University. He is working in the Plant Pathology Section under the direction of Mr. Marsh, and is investigating the morphology of cankers in apple wood caused by *Gloeosporium perennans*.

We regret to record the deaths during the year of two members of the Governing Bodies of the Station : Professor F. Blakemore, who had represented the Senate of the University on the Agricultural Committee since 1950, and Mr. G. A. Nott, a representative of the Worcestershire County Council on the Governing Body of the National Fruit and Cider Institute. Mr. Nott had long been actively associated with the work of the Institute through his business activities in fruit growing and cider and apple juice manufacture. He had co-operated with the Station in its cider orcharding schemes and had also served as a member of the Agricultural Research Council Committee concerned with the Station programme of research on cider and fruit juices.

It is pleasing to record the award of the B.E.M. to Mr. F. E. Maltby, Chief Laboratory Steward of the Institute. Mr. Maltby has a long record of service at Long Ashton, having joined the staff in 1914. He has held his present post of high responsibility since 1936, and throughout his service he has devoted his energies wholeheartedly to the work of the Station.

There were two retirements of note from the Agricultural Committee during the year : Mr. J. H. Alpass, C.B.E., and Professor MacGregor Skene. Mr. Alpass had been the representative of the Gloucestershire County Council since 1941. During his service on the committee Mr. Alpass has always taken a lively interest not only in committee matters but in the research work and other activities of the Station. He has been a familiar figure at the meetings of the National Fruit and Cider Institute and at our annual field days. We trust that he will continue to attend our field days and our annual cider sampling days at which he will always be assured of a warm welcome.

Professor Skene, who had been a representative of the Senate of the University on the committee for many years, retired from the committee on July 31st on his retirement from the Chair of Botany in the University. He had also been a member of the Finance and Business Sub-Committee for a long period, and in this capacity had given much useful service to the Station. Professor Skene had long taken an active interest in the work of the Station,

and in the days when the Department was also responsible for the advisory work in agriculture and horticulture for the Bristol Province, he had acted as the Advisory Centre's unofficial consultant on botanical problems, and particularly on those problems involving the characters and identification of grasses and uncommon weeds. For the Long Ashton Station he served regularly on the staff appointments committees, often as chairman, and on these his shrewd judgment of scientific ability and personal qualities has contributed much to the recruitment of the present scientific staff. As Director I am grateful for this opportunity to place on record my personal thanks and the thanks of the Agricultural Committee to Professor Skene for his long and valued services to the Department. Members of the Agricultural Committee and the Station staff tender to Professor and Mrs. Skene their best wishes for a long and happy retirement.

Professor Skene's place on the Agricultural Committee and the Finance and Business Sub-Committee has been filled by Professor J. E. Harris, to whom we extend a hearty welcome. We trust he will find much to interest him in the work of the committees.

Staff.

The following staff changes occurred during the year :—

Appointments to scientific staff.

Dr. R. A. Webb, who was appointed to the A.R.C. Unit of Plant Nutrition in 1953, whilst in the employ of the Government of The Gambia, took up residence at Long Ashton on 1st June, 1955. Details of Dr. Webb's previous training and service are given in the 1953 Report.

Dr. E. J. Skerrett was appointed to the Analytical Section of Insecticides and Fungicides Chemistry from 1st May, 1955, to succeed Mr. G. V. Coles. Dr. Skerrett is not a newcomer to Long Ashton as he worked in our laboratories from 1949 to 1952 as a postgraduate student for the degree of Ph.D. of Bristol University. Since 1952 he has held a research appointment in industry. We are pleased to have Dr. Skerrett once more with us to open up the new fields of work in our recently established Section of Analytical Chemistry.

Mr. T. E. Cobbald was appointed from 1st August, 1955, to one of the posts made available in the Entomology Section under the scheme of the Colonial Office. Prior to joining our staff Mr. Cobbald has had a varied experience. On leaving school he first took up practical farming and then turned his attention to engineering, which eventually led to his election as G.I.Mech.E. After this training and experience he entered Wye College as a student of agriculture, and in 1951 graduated B.Sc.(Agric.) in the University of London. His training period in these subjects was broken by service in the Royal Corps of Signals from 1945 to 1948, during which period he served abroad in Greece, Egypt and Palestine. After leaving Wye, Mr. Cobbald joined the staff of the Western Regional Production Development Board of Nigeria, and with this organisation he gained experience in the growing of a wide range of tropical crops. As a member of the Long Ashton staff Mr. Cobbald will be concerned with technical problems relating to the control of pests and diseases of tropical crops, and for this work his previous training and experience should prove of great value.

Mr. E. A. Fribbins was appointed to the post of Workshops Engineer from 1st February, 1955, also under the scheme of the Colonial Office. Mr. Fribbins's previous service has been entirely with the Post Office, which service he joined in 1936. His early years were spent in the Department's research laboratories at Dollis Hill, where he worked on the development of optical equipment and high vacuum apparatus. In 1940 he was transferred to the G.P.O. Radio Laboratories, Backwell, Somerset, where he had a wide and varied experience in metal work, and the running and servicing of machines and the use of specialised equipment and tools. Mr. Fribbins holds the City and Guilds Certificate in Radio and Technical Electricity.

Miss J. Sherborne was appointed as Bacteriologist in the Domestic Food Preservation Section from 1st August, 1955, to succeed Miss S. J. Perkins. Miss Sherborne, who is a graduate in Botany (B.Sc.Hons.) of the University of Bristol, had previously been employed in the Domestic Food Preservation Section as an Assistant Experimental Officer.

Mr. D. Wilson was appointed as Plant Breeder in the Pomology Section from 1st October, 1955. Mr. Wilson graduated B.Sc.Hons. in Agricultural Botany at King's College, University of Durham, in 1952, after which he spent three years of postgraduate study in genetics and cytology under Professor P. T. Thomas at the University College of Wales, Aberystwyth. Mr. Wilson carried out his national service training in the Royal Air Force from 1947 to 1949. He fills the post vacated by Mr. Spinks on his retirement in 1953.

Appointments to assistant staff.

Miss M. E. Holgate, Assistant Experimental Officer, was appointed in the Statistics Section from 1st January, 1955. Miss Holgate is a graduate in Mathematics of Oxford University and had subsequently held appointments with firms engaged in the aircraft industry.

Mr. D. R. Clifford, Assistant Experimental Officer, was appointed in the Organic Chemistry Section, from 1st January, 1955. Mr. Clifford has had previous experience in the tar distillation industry. He served his period of national service in the Royal Air Force.

Mr. G. V. C. Davies, Assistant Experimental Officer, was appointed as greenhouse officer in the Nutrition of Fruit Plants Section, from 4th April, 1955. The pot experimental work on this subject has been in abeyance since 1939 and Mr. Davies has been appointed to resume these experiments. He is a graduate, B.Sc. (Hons.) Botany, of the University of Bristol, and since graduating has completed his national service in the Royal Corps of Signals.

Mrs. J. M. Bowcott, Assistant Experimental Officer, was appointed in the Domestic Food Preservation Section, from 10th October, 1955, to succeed Miss J. Sherborne. Mrs. Bowcott is a B.Sc. in Chemistry of Reading University, and also holds a postgraduate diploma in Bacteriology of that University. She has had previous experience in industry and at the Edinburgh and East of Scotland College of Agriculture and the Low Temperature Research Station, Cambridge.

Miss T. Goodman, Assistant Experimental Officer, was appointed to the Unit of Plant Nutrition from 1st October, 1955. Miss Goodman graduated in Botany, B.Sc.(Hons.) from the University College of the South West, Exeter, in 1955.

Mr. J. H. Scawin, Assistant Experimental Officer, was appointed to the Unit of Plant Nutrition from 1st October, 1955. Mr. Scawin, who has recently been awarded the A.R.I.C. has had previous experience at Ministry of Supply Research Establishment centres.

Mr. D. P. Hucklesby, Assistant Experimental Officer, was appointed to the Unit of Plant Nutrition from 1st December, 1955. Mr. Hucklesby is a graduate of King's College, University of London, B.Sc.(Hons.) Botany, and previous to his college training he was employed as an assistant in the Plant Pathology Department of Rothamsted Experimental Station.

Mr. H. M. Webber, Assistant Experimental Officer, Unit of Plant Nutrition, returned from special leave extending from 1st October, 1954, to 20th June, 1955.

Miss B. Bird, Assistant (Scientific), Entomology, from 1st January, 1955, to succeed Mr. G. Bracey.

Miss M. Waller, Assistant (Scientific), Mycology, from 3rd January, 1955, to succeed Miss Waugh (promoted).

Mr. D. M. James, Assistant (Scientific), Unit of Plant Nutrition, from 1st January, 1955, to succeed Mr. Graham Jones.

Mr. B. L. Davies, Assistant (Scientific), Cider and Fruit Juices Section, from 21st March, 1955, to succeed Miss Y. P. May.

Mr. N. T. Weatheritt, Assistant (Scientific), Pomology, from 21st March, 1955, to succeed Mrs. R. Lewis.

Miss P. J. Davey, Assistant (Scientific), Domestic Food Preservation, from 1st June, 1956, to succeed Miss M. Hooper.

Miss H. A. Clarke, Assistant (Scientific) (Temporary), Nutrition of Fruit Plants, from 3rd October, 1955.

Miss G. North-Smith, Assistant (Scientific), Cider Pomology, from 1st October, 1955.

Mr. B. V. Palmer, Assistant (Scientific), Unit of Plant Nutrition, from 26th September, 1955, to succeed Miss R. A. Paget.

Miss K. A. Taylor, Assistant (Scientific), Unit of Plant Nutrition, from 1st October, 1955.

Mr. J. T. Davies, Assistant (Scientific), Physical Chemistry, from 1st November, 1955.

Miss D. I. Conibear, Assistant (Scientific), Entomology, from 1st November, 1955.

Resignations : Scientific staff.

Mr. G. V. Coles, Analytical Chemistry Section, from 31st January, 1955, on appointment to a post in the Colonial Service in Uganda.

Dr. W. Plant, Unit of Plant Nutrition, from 31st March, 1955.

Miss S. J. Perkins, Domestic Food Preservation Section, from 1st July, 1955.

Miss J. Sherborne, Domestic Food Preservation Section, from 30th November, 1955.

Resignations : Assistant staff.

Mrs. O. M. James, Assistant Experimental Officer, Unit of Plant Nutrition, from 31st January, 1955.

Mr. D. W. Burd, Assistant Experimental Officer, Pomology Section, from 31st December, 1955.

Mrs. R. Lewis, Assistant (Scientific), Pomology, from 31st March, 1955.

Miss R. A. Paget, Assistant (Scientific), Unit of Plant Nutrition, from 13th August, 1955.

Miss C. C. Byrne, Assistant (Scientific), Domestic Food Preservation Section, from 30th September, 1955.

Mrs. B. Merchant, Assistant (Scientific), Nutrition of Fruit Plants, from 30th November, 1955.

Administrative staff : appointments.

Miss A. P. Winters, Clerical Assistant, from 14th March, 1955, to succeed Mr. D. W. Godfrey.

Miss D. A. Pearce, Junior Clerical Assistant, from 9th August, 1955, to succeed Miss Winters, promoted.

Administrative staff : resignations.

Mr. D. W. Godfrey, Clerical Assistant, from 16th February, 1955, on National Service.

Miss P. J. Roe, Typist/Telephonist, from 16th February, 1955.

Visits Abroad.

Dr. H. G. H. Kearns, on behalf of the Colonial Office, visited Ulm, Germany, from 23rd to 30th January, 1955, to inspect fire escape apparatus considered suitable for the examination of the leaf crowns of coconut palms. The apparatus consists essentially of an extending ladder, with platform, the whole being mounted on a transport vehicle from which it is operated. The model has been subsequently purchased and is now in use in investigations in Zanzibar.

Dr. Kearns also visited Jamaica from 28th November to 23rd December, 1955, to inspect the results of spraying trials against Leaf Spot of banana.

Dr. R. A. Webb visited The Gambia from 11th October to 7th November, to assess the results of the fertilizer field trials laid down in extension of his pot experiments.

The Director took part in a symposium on the subject of " Problems of the Oligoelements in Vegetable and Animal Life," organized as a Study Week by the Pontifical Academy of Sciences, and held in the Academy Buildings in the Vatican City from 24th April to 1st May, 1955. The participants included leading workers in the subject from several European countries, and also one from the U.S.A. A pleasant feature of the meetings was the presence of Professor Gabriel Bertrand, one of the original workers in the field of trace elements research, whose first results, on the essential nature of manganese for plants, were published in 1905. The proceedings of the symposium will be published by the Academy, and will include as an introductory paper an account by Professor Bertrand of his early work.

The Director also spent the period May 8th 23rd as a Visiting Lecturer in the Hochschule für Bodenkultur, Vienna, under the Foreign Universities Interchange Scheme. The visit was made at the invitation of the Austrian Minister of Education, and sponsored by The British Council.

Dr. D. J. D. Nicholas attended, by special invitation, an International Congress at Rudesheim and Giessen, Germany, organized by the Federal Board of Nutrition, Agriculture and Forestry of Western Germany. He contributed a paper to the conference entitled " Pathological effects of deficiencies and excesses of minor elements on crop plants."

Dr. L. C. Luckwill returned to Long Ashton from his period as Visiting Professor in Pomology at Cornell University, U.S.A., on 1st August.

Dr. L. C. Luckwill, Dr. C. Bould and Mr. D. L. Abbott represented the Research Station at the XIVth International Horticultural Congress held at Scheveningen, the Netherlands, during the period 29th August–6th September, 1955.

Dr. D. Woodcock attended the XIVth International Congress of Pure and Applied Chemistry in Zurich from 21st to 27th July, 1955.

Degrees, Honours, etc.

Mr. F. E. Maltby was awarded the British Empire Medal in the Birthday Honours list, June, 1955. The medal was presented by Lord Hylton, the Lord Lieutenant of Somerset, at Ammerdown House, Radstock, on 15th September, 1955.

The degree of Ph.D. was awarded to the following during the year :

Mr. S. H. Bennett (Bristol University) for a thesis entitled " Systemic Insecticides."

Mr. S. T. C. Wright, Boots Scholar from 1951 to 1953 (Bristol University), for a thesis entitled " A chromatographic study of auxins in relation to fruit morphogenesis and fruit drop in black currant (*Ribes nigrum*)."

Mr. A. T. K. Corke (Edinburgh University) for a thesis entitled " Some studies in forest and agricultural mycology."

Messrs. J. F. Harris, J. H. Scawin, A. J. Sims and H. M. Webber, all members of the assistant staff, were successful in the A.R.I.C. examination of the Institute of Chemistry. Mr. Webber also gained the B.Sc. (Special) degree of the University of London.

Mr. D. W. Burd, of the Pomology Section, was awarded the N.D.H.

The Director delivered the 8th Amos Memorial Lecture at the East Malling Research Station on 24th November, 1955. The subject of the lecture was " The mineral nutrition of fruit crops—a retrospect."

Buildings and Land.

Work on the greenhouse block, and the laboratories to accommodate the biochemical work of the A.R.C. Unit of Plant Nutrition and the analytical team to investigate methods of analysis of insecticides and fungicides, was begun in early January and has made steady progress throughout the year under favourable weather conditions. The work so far has progressed according to the time schedule, and it is hoped that the buildings will be ready for occupation during the summer of 1956.

Work has continued on the plans for the proposed new laboratory and office block, and for the building to provide accommodation for conferences and canteen and common room requirements. Discussions on the preliminary drawings were held with members of the A.R.C. staff during the summer,

following which detailed drawings have been made by the architect and submitted to the Council for their further consideration.

Several minor building projects were also undertaken during the year, including a small acid fume chamber to adjoin the sand purification room, a block of lavatories for the plantation staff on the north side of the road and adjacent to the workshop stores, and the provision of doors and gates for the farm buildings and workshops blocks.

On the farm the shortage of land that has not hitherto been under fruit crops continues to present difficult problems in the planning of new projects. During 1955 the position as regards suitable land for nursery work has been particularly acute and led to an unsuccessful attempt to negotiate the purchase or rental of land adjoining the farm.

The only alternative to acquiring new land is to speed up the clearing of plantations that have served their original experimental purposes, but this unfortunately entails the use of land not properly " rested " before replanting, and often the removal of trees and bushes that would otherwise remain for cropping purposes.

To attempt to meet our needs in the immediate future we have thus been compelled to accelerate our clearing programme. During the year the following clearing operations were carried out.

In the plantations on the south side of the main Bristol-Weston road : the whole of the black currant area ; the " Hatton " plot, comprising Bramley, Worcester and Lane trees, originally used in the first apple rootstock trial planted in 1920 ; and a section of the pear rootstock and pruning plot. In addition an irregular hedge line separating the arable plantations from the Hillside cider orchard has been cleared and filled in, and a new hedge line established on the orchard side of the old hedge. This operation will extend slightly the area available for experimental plots. On the north side of the farm approximately one acre of the Pathology plot was cleared to prepare for future replanting.

Other Activities.

Members' Day.

The annual Field Day for Members of the National Fruit and Cider Institute was held on 20th July, 1955, on the occasion of the Annual Meetings of the Members and Governors. The main demonstrations illustrated the grassing-down of young apple trees, frame-working and top-grafting of apples, thinning trials with naphthylacetamide, spraying operations on dwarf pyramids and the rooting of gooseberry cuttings.

Cider Sampling Day.

Annual Cider Sampling Day was held on 5th May, 1955, and was attended by some 120 visitors. The morning session opened with the tasting of a series of perries followed by three series of ciders. The latter included ciders made from dessert varieties, from vintage cider varieties of established reputation, and others thought worthy of wider propagation. Ciders demonstrating the effects of fermentation treatments upon cider quality aroused interest among the cider makers present. In the afternoon other work of the section was on

view and a further selection of perries, ciders, non-alcoholic ciders and juices was exhibited. Plantation tours were arranged for the demonstration of top grafting in a cider orchard and of routine spraying operations.

Visitors.

The number of organized parties visiting the Station was 53 compared with 68 in 1954. Three sections of the British Association were entertained : other notable visitors included a party of French growers led by M. Bonnicel of the Paris organization B.E.S.T., a group of German research workers and farmers under the leadership of Professor Loewel of the Horticultural Research Station, Jork, Hamburg, members of the Plantations staff of East Malling Research Station and of the staff of the National Vegetable Research Station, Wellesbourne.

Organized Parties, 1955.

Abbey School, Malvern Wells, Science Students.
B.E.S.T., Paris (Bureau d'Etudes de Standardisation et de Technique des Producteurs de Fruits de Qualité).
Birmingham Science Mistresses.
Birmingham University, Honours Botany Students.
Blue School, Wells.
Bristol Aeroplane Company, Aces Club.
Bristol College of Technology.
Bristol Co-operative Society, Retired Employees.
Bristol Royal Infirmary, Medical Students.
Bristol Taxation Department.
Bristol University, Employees' Association.
Bristol University, Horticultural Students.
Bristol University, Microbiology Students.
Bristol University, Public Health Class.
Bristol and West Council for Aid to Refugees.
British Association, Agriculture Section.
British Association, Botany Section.
British Association, Physiology Section.
British Broadcasting Company, Agricultural Talks Producers.
Cardiff Gardening Association.
Cannington Farm Institute.
City of Bath Training College.
Clifton High School for Girls.
Colombo Plan Trainees.
Diocesan Training College, Barrow Court.
Domestic Preservation Courses, Fruit and Vegetables (2).
Domestic Preservation Courses, Meat and Poultry (3).
East Malling Research Station, Plantations Staff.
French Productivity Centre.
Gloucester Training College of Domestic Science.
Hamburg Fruitgrowers.
Herefordshire N.F.U.
Hereford Training College.
Horfield Townswomen's Guild.
Kingswood Townswomen's Guild.
N.A.A.S. Analysts.
N.A.A.S. Entomologists.
N.A.A.S. and L.E.A. Officers, Grafting Course.
N.A.A.S. and L.E.A. Horticultural Officers, Refresher Course.
National Vegetable Research Station, Wellesbourne, Principal and Staff.
Reading University, Honours Agricultural Botany Students.
Reading University, Horticultural Students.
St. Andrew's (Bristol) Townswomen's Guild.
Scottish Fruit Trials Committee.
Society of Analytical Chemists.
Somerset Fruit Growers' Club.

Sphalerite Society, National Smelting Company, Avonmouth.
Studley College, Horticultural Students.
Swindon Trades Council.
Waterperry Horticultural School.
Wiltshire Federation of Women's Institutes (2).
Wotton-under-Edge School.

The number of visitors from overseas was the greatest on record. Many were visiting this country after attending the Fourteenth International Horticultural Congress in Scheveningen.

Visitors from Overseas, 1955.

Afghanistan :	Mr. G. S. Cotterell, F.A.O.
Argentine :	Miss Emma Welkerling.
Australia :	Mr. J. H. Arbuckle, Perth, Western Australia.
	Dr. J. E. Falk, C.S.I.R.O.
	Mr. E. O. Gedye, Melbourne.
	Mr. B. N. Jephcott, A.I.B., Alice Springs.
	Mr. R. W. Prunster, C.S.I.R.O., Deniliquin.
	Dr. D. Spencer, C.S.I.R.O., Canberra.
	Dr. W. A. T. Summerville, Brisbane.
	Mr. R. Wetselaar, C.S.I.R.O.
	Dr. C. H. Williams, C.S.I.R.O.
Austria :	Dr. Othmar Nolz, Vienna.
	Mr. F. Weber, Schönbrunn.
Basutoland :	Mr. A. Venn, Research Service.
Belgian Congo :	Mr. H. Broeshart, U.A.C. Ltd.
	Mr. J. D. Ferwerda, U.A.C. Ltd.
Canada :	Mr. H. Andison, Victoria, British Columbia.
	Dr. C. J. Bishop, Kentville, N.S.
	Dr. D. S. Blair, Ottawa.
	Mr. D. E. Caudle, Vancouver Island.
	Dr. R. S. Downing, Summerland, British Columbia.
	Dr. D. V. Fisher, Summerland, British Columbia.
	Dr. R. E. Fitzpatrick, Vancouver, British Columbia.
	Professor C. S. Hanes, Toronto.
	Dr. C. Walker, Morden, Manitoba.
	Dr. R. H. Wallace, Quebec.
	Dr. J. M. Ward, Ottawa.
	Mr L. C. Young, Fredericton, N.B.
Chile :	Mr. G. Donoso, Casolla.
Cyprus :	Dr. P. A. Loizides, Nicosia.
Denmark :	Mr. A. Andersen, Copenhagen.
	Dr. Blangstrup Jørgensen, Esbjerg.
	Mr. G. Nielsen, Odense.
Egypt :	Professor M. N. Hashad, Cairo.
France :	Monsieur R. Auclair, Longueville, and his two sons.
	Monsieur J. Billeau, Rouen.
	Monsieur A. Camut, La Lande, Eure.
	Count B. de Cherisey, Paris.
	Messieurs Philip and Pierre de Cherisey, Paris.
	Dr. G. Morvan, Versailles.
	Monsieur A. Moyse, Laboratoire de Botanique de la Faculté des Sciences, Paris.
	Dr. P. Prévot, Paris.
	Monsieur R. Prieur, Le Catelier, and his son.
	Monsieur J. C. Redlich, Gargenville.
	Monsieur Lucien Rollin, Bazainville.
The Gambia :	Sir Percy Wyn-Harris, Governor and Commander-in-Chief of The Gambia, Bathurst.
Germany :	Dr. G. Kemmler, Hanover.
	Mr. H. V. Klämbt, Bonn.
	Dr. E. Raadts, Hamburg.
Gold Coast :	Mr. H. R. Mapother, W.A.C.R.I., Tafo.
	Mr. A. D. McKelvie, W.A.C.R.I., Tafo.
	Dr. D. J. Taylor, W.A.C.R.I., Tafo.

Holland :	Ir. H. Burgmans, The Hague.
	Dr. J. Doorenbos, Wageningen.
	Ir. J. H. van Emden, Wageningen.
	Mr. J. van den Ende, Naaldwijk.
	Dr. H. J. de Fluiter, Wageningen.
	Dr. C. J. Gorter, Wageningen.
	Dr. J. Grosjean, Wageningen.
	Ir. G. W. van der Helm, Amsterdam.
	Dr. J. O. ten Houten, Wageningen.
	Ir. D. Kers, The Hague.
	Dr. E. van der Laan. The Hague.
	Dr. T. H. Thung, Wageningen.
India :	Mr. F. B. Carbasius Weber, F.A.O., Lucknow.
	Dr. K. M. Pandalai, Ochira, South India.
	Mr. Shambhu Man Singh, Nepal.
Israel :	Mr. M. Ben-Dror, Givath Hamoreh.
	Dr. H. N. Plaut, Haifa.
	Dr. P. Spiegel, Rehovat.
Italy :	Dr. E. Baldini, University of Florence.
	Dr. F. Scaramuzzi, University of Florence.
Jamaica :	Mr. C. G. Jordine, Department of Agriculture.
Kenya :	Dr. R. M. Nattrass, Scott Laboratories.
Lebanon :	Mr. G. A. Lundeen, Beirut.
Malaya :	Dr. U. A. Aziz, Singapore.
	Dr. E. W. Bolle-Jones, Rubber Research Institute, Kuala Lumpur.
	Mr. G. R. Fish, Malacca.
	Dr. A. Newsam, Rubber Research Institute, Kuala Lumpur.
Mauritius :	Mr. S. Feillafé, Reduit.
New Zealand :	Dr. R. M. Allison, Christchurch.
	Mr. J. D. Atkinson, D.S.I.R., Auckland.
	Dr. R. W. Bailey, Hamilton.
	Mr. G. A. Hamilton, Lumsden.
	Dr. W. M. Hamilton, Secretary, D.S.I.R.
	Mr. L. Hobbis, Auckland University College.
	Mr. K. C. Hockey, Massey Agricultural College.
	Mr. and Mrs. N. H. Jefferis, Invercargill.
	Dr. A. I. Johns, D.S.I.R., Palmerston North.
	Mr. J. E. Kelynack, Timaru.
	Miss P. Kummert, Dunedin.
	Mr. M. O'Connor, Scientific Liaison Officer.
	Mr. D. A. Stewart, Levin.
Nigeria :	Mr. S. A. Adeniyi, Department of Agriculture.
	Mr. J. A. Ayorinde, Ibadan.
	Mr. J. A. R. Bates, Ibadan.
	Mr. Raymond Bull, W.A.I.F.O.R., Benin City.
	Mr. T. M. Greensill, Department of Agriculture.
	Mr. C. W. Hartley, W.A.I.F.O.R., Benin City.
	Mr. G. M. Higgins, Department of Agriculture.
	Mr. P. T. Nelson, Department of Agriculture.
	Mr. C. O. Phillips, Department of Agriculture.
	Mr. P. B. Tinker, W.A.I.F.O.R., Benin City.
	Mr. V. J. Weston, Department of Agriculture.
North Borneo :	Mr. C. Hunter.
Norway :	Mr. O. Hvatum, Vollebekk.
	Professor G. Hygen, Vollebekk.
Pakistan :	Mr. S. A. Aga, Baluchistan.
Poland :	Dr. S. A. Pieniazek, Skierniewice.
Rhodesia :	Mr. C. B. Payne, Rhodes Orchards.
South Africa :	Dr. A. S. Ashworth, Johannesburg.
	Mr. R. L. Bridgman, Elgin.
	Professor and Mrs. E. E. A. Gledhill, Rhodes University.
	Dr. F. X. Laubscher, Stellenbosch.
	Dr. A. J. van der Merwe, Pretoria.
	Dr. H. L. Pearse, Rustenburg.
	Dr. W. A. K. Stubbings, African Explosives.
	Dr. Vincent A. Wager, Duban.
	Commander R. B. Wilson, Johannesburg.
Spain :	Miss Maria Antonia Gonzales, Madrid.
	Miss Elena Garcia de Dios, Madrid.

Sweden :	Dr. B. Hansen, Lund.
Switzerland :	Dr. R. Christ, Basel.
	Mr. J. Julen, Valais.
	Mr. G. Perraudin, Lausanne.
	Mr. H. Roshur, Saillon.
	Dr. and Mrs. E. Züllig, Wädenswil.
Tanganyika :	Mr. L. J. Hart, Coffee Research Station.
Tasmania :	Dr. L. W. Miller.
Thailand :	Mr. Pue Rochanapurananda, Bangkok.
	Mr. Vichian Sakoramonkol, Bangkok.
Trinidad :	Mr. G. Havord, I.C.T.A.
	Dr. G. A. C. Herklots, I.C.T.A.
	Mr. T. A. Jones, I.C.T.A.
Turkey :	Dr. Mehmet Dokuzoguz, Ankara.
	Miss H. Ismen, Adapazari.
U.S.A. :	Dr. C. L. Burkholder, Purdue Experiment Station, Indiana.
	Dr. F. P. Cullinan, Beltsville.
	Dr. E. G. Fisher, Cornell University.
	Dr. J. R. Furr, Date Gardens, California.
	Dr. E. R. Glass, Geneva, New York.
	Dr. L. V. Hall, Public Health Department.
	Dr. R. W. Holley, Geneva, New York.
	Dr. C. M. Johnson, University of California, Berkeley.
	Professor Gordon Mackinney, University of California, Berkeley.
	Mr. E. F. Oatman, Yonkers, New York.
	Mr. Bruce Oatman, Yonkers, New York.
	Dr. M. Potgieter, University of Connecticut.
	Professor and Mrs. E. L. Proebsting, University of California, Davis.
	Dr. Ralph Shay, Purdue University, Indiana.
	Professor P. A. Stout, University of California, Berkeley.
	Professor H. B. Tukey, Michigan.
Uruguay :	Mr. D. Pellegrino.
Venezuela :	Dr. R. C. Tincknell, Caracas.
Yugoslavia :	Professor B. Vovk.
Zanzibar :	Mr. Ahmed Aley.
	Mr. and Mrs. A. K. Briant, Department of Agriculture.

Shows and Demonstrations.

Exhibits were staged at the following shows :—

Bath and West and Southern Counties Society, Launceston, 1st–4th June.
Royal Horticultural Society, Chelsea, 24th–27th May.
The Royal Agricultural Show, Nottingham, 5th–8th July.

Students and Voluntary Workers.

The number of students whom it has been possible to accommodate during the year has again been small, owing to inadequate bench space, and we have not been able to offer the facilities we would wish to do to those who have worked with us. It is hoped, however, that these conditions will be improved when new buildings are erected in the next year or two.

Miss F. L. C. Blackwall obtained a year's extension to her Ministry of Agriculture post-graduate scholarship in Husbandry, and is continuing for a further year her work in the Pomology Section.

Mr. J. M. Rayner completed his tenure of the Boots Scholarship at the end of September 1955, and was succeeded by Mr. R. O. Sharples who, as mentioned earlier, is investigating a problem in the Plant Pathology Section.

Mr. R. G. Cove completed his period of training in March whilst holding the Cider Memorial Studentship. The studentship has not been awarded since that date as no suitable candidate has been forthcoming.

Mr. Shambhu Man Singh of Nepal, India, a student under the Government Scheme for Technical Co-operation in South and South-East Asia, spent the month of July working in the nursery under Mr. Hobbis and assisting with the growth-substances experiments in the Pomology Section. Mr. Shambhu Man Singh had been an assistant in the Pomology Section at East Malling Research Station for the year previous to his Long Ashton visit.

Dr. R. Comber, who holds a Colonial Agricultural Research Studentship, commenced a period of training under Dr. R. A. Webb, in August, preparatory to service in The Gambia as a Plant Physiologist on the Colonial Office staff.

Mr. R. A. Bull of the West African Institute for Oil Palm Research, Benin City, worked in Dr. Hewitt's Section from 13th June to 14th September before returning to W.A.I.F.O.R. to set up a unit of plant nutrition there.

Dr. Elwood G. Fisher, Associate Professor, Department of Pomology, Cornell University, New York, as mentioned above, worked in the plant nutrition laboratory from September 1955–January 1956.

Dr. Antonia Medina, a Plant Biochemist from the Instituto de Edafologia y Fisiologia Vegetal, Madrid, arrived at Long Ashton in October 1955 to spend a year working under the direction of Dr. Nicholas in the Unit of Plant Nutrition, on a problem relating to the enzyme fructokinase. Dr. Medina is the holder of a British Council scholarship.

Social Activities.

The Welfare Committee has this year been under the chairmanship of Miss M. E. Kieser. They were, as usual, responsible for organizing the annual Christmas Party in the Village Hall on the evening of 21st December, when a pantomime was produced by the staff and much enjoyed by all who attended.

The Christmas Lunch, arranged by the Canteen Committee, took place on the following day, and was a particularly successful occasion this year, when we were very happy to have our American visitors, Professor and Mrs. Proebsting, and Dr. and Mrs. Fisher, as our guests.

The Cricket Club again enjoyed a comparatively successful season, with membership well maintained. Six matches were won and nine lost; the Club again reached the last sixteen in the Bristol Evening Post H.E.R. Cup. The highlight of the season was the fixture with the National Vegetable Research Station, Wellesbourne, played in Bristol, which Long Ashton won after a very close game.

The Tennis Club had a very successful season, the Men's team doing particularly well. They were the winners of Group V of the Bristol League, winning eight of the ten matches played, losing one and drawing one. As a result the Club has been promoted to Group IV. The Ladies' Team played only three matches, of which they won two. Eleven friendly matches were arranged during the season, of which five were won, four lost and two drawn.

Acknowledgments.

We welcome this opportunity to express our sincere thanks to those members of the Station staff who have carried out special duties during the year in addition to their normal work. Dr. Kearns has continued his much valued help in the planning of new buildings and in superintending the work carried out in the Station workshops, including repairs to equipment, machinery

and tools for laboratories and farm. Mr. Spinks has continued as Librarian. Miss Kieser has served as Chairman of the Welfare Committee, and Mr. Burroughs and Mr. Stott as Chairmen of the Canteen Committee. We are grateful to them and to Miss Locke, Miss Perkins, Miss Sherborne, Miss Cousin, Miss Jackson and Miss Sturdy, who have all helped with the organization of meals during the course of the year.

Our best thanks are also due to all other members of staff for their valued services to the Station during the year, and for the contributions each has made towards the fulfilment of the research programme.

It is a special pleasure to acknowledge the help of our many friends outside the Station, particularly those growers and commercial firms who have assisted us in our work during the year by the provision of gifts of materials and facilities to carry out experiments.

SUMMARY OF RESEARCH, 1955.

Pomology and Plant Breeding.

Plant Breeding and Systematic Pomology.

Following the appointment of a plant breeder a start has been made in assembling a working collection of *Ribes* species which will be surveyed as potential breeding material for improving black currant varieties. 60 species have so far been obtained from sources in this country and abroad. Investigation of a large number of gooseberry varieties has revealed much confusion in nomenclature and an attempt is being made to clarify the classification. The situation is further complicated by the presence of widespread virus infection in almost all varieties. Pilot trials with heat therapy treatment have been carried out in co-operation with East Malling, but the final results will not be known for another season.

Growth substances.

The first trials with NAm (1-naphthaleneacetamide) as a thinning agent for apples show this compound to be less damaging to foliage and more promising as a thinning agent than NAA (1-naphthalene acetic acid). On Crawley Beauty, 25 p.p.m. applied four days after petal-fall considerably increased the saleable crop of apples (p. 59). Chemical thinning trials with plums, using NAA and 3 Cl-IPC (*iso*propyl N-(3-chlorophenyl) carbamate) applied one month after bloom, were not so successful: no significant thinning was obtained with any of the concentrations used and, at the higher rates of application, there was considerable inhibition of extension growth and yellowing of the foliage which persisted throughout the season.

A comparison of NAA, 2:4:5-T (2:4:5-trichlorophenoxyacetic acid), and 2:4:5-TP (α -(2:4:5-trichlorophenoxy) propionic acid) for reducing the pre-harvest drop of dwarf pyramid trees of Worcester Pearmain showed that, at 10 p.p.m., NAA was approximately twice as effective as the phenoxy compounds on this variety. This is at variance with American results which indicate that, on most varieties, the phenoxy growth substances have a longer period of effectiveness than NAA.

The effect of relatively high concentrations of 2:4:5-TP in hastening the maturity of Worcester Pearmain has already been reported (Report for 1953). During 1955 observations were made on the residual effects of this treatment on trees sprayed with 40 p.p.m. during 1954. The damage was of the same type, though much less severe than that which occurred on the trees sprayed in 1953 (Report for 1954). No carry-over effect was observed on fruit size or maturity, though the total crop weight was reduced to approximately 67% of that of the untreated trees. Unless ways can be found of reducing this residual damage it will not be possible to recommend the use of this compound as a fruit ripening agent for Worcester.

Experiments in which the seeds are removed from growing apple fruits at different stages of development have been continued during the past year. Treatment with NAA in lanolin failed to stimulate further growth of young fruitlets after the removal of the seeds. From 5 weeks after petal-fall onwards,

however, the growth of the fruits appeared to be increasingly independent of the seeds, and many fruits continued to develop without seeds, both in the presence and absence of added growth-substance.

Chromatographic techniques were applied to the study of the natural hormones of the apple leaf. In the acidic fraction of leaf extracts three auxins and two growth inhibitors were found that appear to correspond with those already found in the seed. No evidence was obtained of the presence of indoleacetic acid in the leaf tissue, indicating that this auxin may not be of such universal distribution as is commonly supposed. The application of similar techniques to a study of growth hormones in tomato ovaries and fruits has so far failed to reveal any consistent auxin pattern. The only constant feature of these chromatograms is a growth-inhibiting substance with an R_F value of 0·5–0·6 in butanol/ammonia which may be identical with that obtained from apple.

Laboratory studies with anti-auxins have shown that the action of these substances on root initiation and petiole abscission may be quite different from their well-known effects on cell extension growth. Thus, *trans*cinnamic acid, which in cell extension tests reduces the activity of NAA, has been shown to act synergistically with this compound in delaying abscission of Coleus petioles. Similarly, coumarin, a well-known growth inhibitor, has been found to stimulate root initiation and delay petiole abscission at relatively high concentrations. Thiourea and 2:3:5 tri-iodobenzoic acid, which in some plants are effective in breaking apical dominance, and the growth inhibitor maleic hydrazide, proved ineffective in stimulating " feathering " on maiden trees of Worcester Pearmain.

In co-operation with the Organic Chemistry Section investigation of the relationship of molecular structure to biological activity in a series of compounds related to 2-naphthoxyacetic acid was continued (see Organic Chemistry, p. 30).

Dormancy. On the assumption that low temperature may alter the enzyme balance of dormant apple seeds so as to favour the breakdown of storage products to soluble constituents available for growth, attempts have been made to break dormancy by soaking excised dormant embryos in solutions of various simple metabolites, vitamins and growth-substances separately and in various combinations for 1, 3 and 5 days at 5°C. prior to incubation at the neutral " compensation " temperature of 17°C. None of these treatments increased the germination capacity of the embryos. A paper on " Temperature and the dormancy of apple seeds " was presented at the XIVth International Horticultural Congress at Scheveningen by D. L. Abbott.

Variety Trials and Cultural Experiments.

The final report on the cropping of six varieties of black currant planted 1944 -5 will be found on p. 61. It is suggested that the economic life of a black currant plantation, hitherto assumed to be 10 years, can be confidently raised to 15 years under present-day methods of management. The new black currant variety plot, consisting of 11 named and 5 seedling varieties, is now well established and is expected to produce its first crop in 1956.

The experiment on the growth and cropping of apple trees under different cultural systems has now completed its eighth year. Trees of Cox, Superb and Winston on M.IX withstood the summer drought condition much better than did the same varieties on M.II. This difference was reflected in the almost

total absence of leaf-drop from trees on M.IX during August and September, whereas leaf-drop from trees on M.II was very marked. The fruit size of trees on M.IX was also greater than that of the same varieties on M.II.

The tree-shaping and pruning trial with pears, started in 1942, was completed during 1955. An unexpected development has been the scion rooting of many of the trees on Quince C which, as a consequence, have grown larger than those worked on Quince A.

On the plum plot laid down in 1948 severe casualties have been suffered from bacterial canker, the losses ranging from nil in Warwickshire Drooper to 65% in Jefferson Gage on Myrobalan B.

Of six varieties of peach on Brompton stocks planted in 1948, only Rochester has cropped with any degree of regularity. Winter killing of the shoot tips has been severe on all the varieties.

Rootstocks and Propagation.

Observations were continued on trees of Cox, Worcester and Lambourne worked on apomictic seedling stocks. The oldest trees on the Station have completed their third year of growth in the nursery and will now be transplanted to a permanent plot for further observation. Trees on *Malus sikkimensis* carried their first substantial crop in 1955, whereas few fruits were formed on the trees on the more vigorous *M. toringoides*. This has further accentuated the size difference between the trees worked on these two stocks (see Report for 1953).

Various degrees of incompatibility between perry pear varieties and Quince A rootstocks have been encountered and efforts are being made to overcome these by double working with Beurré Hardy.

A selection of vigorous cider varieties and Crab C have been tested for their suitability as stembuilders for standard trees. Crab C has proved the most vigorous, both on its own roots and when budded on seedling rootstocks (p. 46).

Further work on the distribution of rubbery wood virus in clonal stocks has shown that, in general, the small sized stocks from any particular clone have a higher virus content than the large ones (p. 51). A rubbery wood condition in the variety James Grieve has been transmitted by grafting to Lord Lambourne but an apparently similar condition on Bulmer's Norman proved not to be graft transmissible and was therefore presumably not due to virus infection. The condition known as " flat limb " in the variety Gravenstein has been transmitted by grafting to Lord Lambourne and has been shown to be distinct from the so-called " flat limb " previously recorded on Lord Lambourne in this country. Certain apple varieties listed at p. 55 have been shown capable of acting as symptomless carriers of the vein-banding strain of apple mosaic virus.

In further work on gooseberry propagation the effect of different times of taking and planting cuttings has been investigated and attention has also been given to the source of the cuttings (p. 78). The latter is apparently a very important factor in determining the percentage rooting.

Cider and Perry Varieties.

Work on the revision of the " Recommended List of Cider Varieties for General Planting " proceeded in collaboration with a sub-committee of the

Advisory Committee. An investigation of about 50 lesser known varieties is in progress and it is hoped that some of these will prove suitable for general planting.

A further 237 farms in south Herefordshire were visited during the summer in connection with the survey of perry pear varieties. It is hoped to complete the survey of Herefordshire in 1956. Work on the description of perry pear and cider apple varieties was continued.

Further investigations on the pollination requirements of cider varieties have suggested that the degree of self fertility of certain partially biennial varieties is considerably higher in the " on " than in the " off " years.

An exhibit dealing with the distribution and history of perry pears was staged at Chelsea Flower Show.

Plant Nutrition : Fruit.

Chemical analysis of Royal Sovereign strawberry leaves from plants on the long-term manurial deficiency plots at Long Ashton, and from the nutrition plots at the Experimental Horticulture Station at Rosewarne, have confirmed previous results obtained with the variety Climax : it would appear that the strawberry plant can obtain sufficient phosphorus for normal growth and fruiting from soils low enough in available phosphate to induce severe phosphorus deficiency in many agricultural crops. The strawberry plant needs, however, a high level of available potassium in the soil for healthy growth and high yields of top grade fruit.

The first results from the manurial experiment on Royal Sovereign strawberry plants *under cloches* at Rosewarne were obtained in 1955. From the data so far analysed it would appear that the nutrient status of the runners prior to planting is more important than the fertility of the soil, or the nature and amount of the fertilizers applied. Yields of fruit do not appear to have been affected by any fertilizer treatment. Application of phosphatic fertilizer prior to planting had no significant effect on leaf phosphorus status, even though the soil was extremely low in available phosphate (N/2 acetic soluble P= 0·00035%). Potassic fertilizer increased the leaf potassium at fruiting by more than 50%.

A preliminary investigation has been made on disc versus whole-leaf sampling, prior to a detailed study on the relationship between fertilizer treatment, leaf nutrient status and plant performance of black currant. Disc sampling may prove useful for following seasonal changes in the nutrient status of individual plants without causing any appreciable loss in total leaf area (p. 93).

A new apple manurial experiment has been started at the Efford Experimental Horticulture Station.

Work on iron chelates has progressed along three main lines : analytical methods for estimating chelating agents, the behaviour of iron chelates in soil, and field studies in relation to their control of lime-induced chlorosis in fruit plants.

A new analytical method has been developed, using ultra-violet spectrophotometry, which enables the concentration of chelated iron, and the total amount of chelate in solution, to be estimated quantitatively (27).*

* See *Publications*, 1955, pp. 221–224.

Soil stability tests have been made with a number of iron chelates. Using a highly calcareous soil, the loss of iron from the iron chelates of ethylenediaminetetraacetic acid (Fe-EDTA) and hydroxyethylethylenediaminetriacetic acid (Fe-HEEDTA) was found to be rapid : the initial loss was greater from Fe-HEEDTA, but after 15 days the losses were roughly equal. In less detailed experiments with the iron chelates of di-hydroxyethylethlyenediaminediacetic acid (Fe-HEEDDA), diethylenetriaminepentaacetic acid (Fe-DTPA) and cyclohexylenediaminetetraacetic acid (Fe-CDTA), the two last chelates were found the most stable. The loss of soluble iron when solutions of iron chelates are applied to soil is probably due to two main reactions : sorption by the clay (a small and almost constant amount for a particular soil), followed by a fairly rapid exchange of calcium for iron in the chelate molecule. These laboratory studies emphasize the importance, previously noted in field work, of transporting the iron chelate to the roots in the shortest possible time.

Field experiments described in this Report (p. 87) again showed that a complete recovery from chlorosis, during the year of treatment, can be obtained by soil applications of iron chelates to chlorotic pear trees, provided the chelates are thoroughly watered-in, or placed near the rooting zone by means of liquid sub-soil injection.

Laboratory, pot culture and field tests were made with the iron complexes of lignin sulphonic acid and of several tannins. Although these complexes maintained an adequate supply of iron to plants grown in water culture, even at high pH values, no response was obtained under field conditions when they were applied to chlorotic pear trees growing in a calcareous soil.

In collaboration with the Physical Chemistry Section experiments were made to determine whether isotopic exchange, between ^{59}Fe-labelled iron chelates and soil iron, would invalidate the results from soil-plant iron absorption studies using the radio-isotope technique (see Physical Chemistry, p. 31).

Chemistry of Insecticides and Fungicides.

General and Analytical Chemistry.

Attention has been given to the development of methods for insecticide and fungicide spray deposits, for use in the assessment of the efficiency of spray materials and of the performance of spraying equipment. A rapid method for the determination of surface deposits of copper has been used (p. 98) and the method for captan deposits on fruits and foliage described in the 1954 Report has been improved (p. 103). The principles of the Schechter-Hornstein method for the determination of small amounts of *gamma*-BHC have been examined, and a modified apparatus has been used and interference reduced (p. 108).

An oxidation step is suggested in the method for DDT deposits (1953 Report) to eliminate interference in the analysis of highly-pigmented plant and soil extracts. Penetration of DDT within pea, field bean and cabbage leaves was found, but no movement to the pea and bean seeds or to the developing heart of the cabbage was detected. A technique has been worked out for the determination of DDT, *gamma*-BHC and chlorbenside occurring together in spray deposits, and the behaviour of the three components on foliage has been followed. The method has been used for the determination of DDT and *gamma*-BHC occurring together in soil and grain (p. 106).

An examination has been made of the method of O'Donnell *et al* for the determination of micro-gram quantities of aldrin and dieldrin. The phenyl azide reagent was found to be sensitive to light, necessitating operations in a darkened room, and showed fluctuations in sensitivity from day to day. The method in its present form appeared to be unsuitable for the examination of large numbers of samples. An electronic method for the determination of chlorine in organic insecticides has been investigated with a view to its use in work on spray deposits.

Conjoint work has been carried out with the Entomology Section on the assessment of the efficiency of spraying methods. The distribution of DDT spray deposits in fruit buds and flower clusters subjected to different treatments has been determined chemically. Other analytical work has been concerned with spraying trials on bananas and potatoes and with the persistence of DDT under tropical conditions and in soil.

Earlier work on the effect of copper and sulphur upon fungus catalase has been extended to an investigation of the action of four fungicides upon fungus enzymes (see Nutrition of Micro-organisms, p. 44).

Organic Chemistry.

Investigations into the relationship of the molecular structure of some naphthyloxy compounds and biological activity as plant growth-regulating substances have continued in collaborative work with the Pomology Section. The influence of side-chain length on the activity of ω-(2-naphthyloxy)-n-alkylcarboxylic acids in the induction of parthenocarpy in tomatoes has been reported (29). A preliminary account of the activity of some α-(2-naphthyloxy)-acids and some nuclear substituted 2-naphthyloxyacetic acids was given at the Plant Growth Substances Conference held at Wye College (July 1955). The inactivity in the tomato ovary test of 3-hydroxy-2-naphthyloxyacetic acid in contrast to the high activity of the 3-chloro and parent acids, has emphasized the need for the preparation of further 3-substituted-2-naphthyloxyacetic acids. Syntheses of the 3-methyl and 3-methoxy compounds are in progress, and routes to the unknown 3-nitro-2-naphthol are being investigated.

In an examination of the effect of a long alkyl side-chain on the fungicidal activity of 8-hydroxyquinoline (" oxine "), eleven new analogues have been prepared for test (53). Various new 4-n-alkyl-2-nitrophenols have been prepared during the course of this work. Synthesis of other oxine derivatives is in progress.

Preliminary results of an investigation with the Plant Pathology Section into the metabolism of ω-(2-naphthyloxy)-n-alkylcarboxylic acids by fungi have been described (54). It has been shown, using a replacement technique, that *Aspergillus niger* grown in surface culture converts 2-naphthyloxyacetic and β-(2-naphthyloxy) propionic acids into the corresponding 6-hydroxy derivatives. Higher members of the series $C_{10}H_8O\ (CH_2)_n\ COOH$ have been shown to undergo β-oxidation in addition to 6-hydroxylation. An extension of this work to other series of aryloxyalkyl carboxylic acids and to other fungi is in progress.

Physical Chemistry.

The general behaviour of ^{32}P-labelled demeton-S in plants has been investigated in collaboration with the Entomology Section. Following application to apple, bean and *Coleus* leaves, evaporation, absorption and chemical

degradation of the demeton-S to less volatile, toxic derivatives occurred, effectively removing all demeton-S from the leaf surface within a few hours. Evaporation under glasshouse conditions gave rise to a fumigant effect on *Aphis fabae*. No demeton-S and only very small amounts of toxic degradation products were translocated from treated leaves. Autoradiographic tests confirmed the limited movement in leaves of demeton-S and its derivatives. Following application of demeton-S to bean roots, rapid absorption and translocation of demeton-S and its toxic derivatives occurred. Demeton-S and its toxic derivatives appeared to move more freely in xylem than in phloem (45).

Laboratory investigations in collaboration with Nutrition of Fruit Plants Section with both ^{59}Fe-EDTA and ^{59}Fe-HEEDTA showed that conversion of iron chelate to calcium chelate occurs rapidly in contact with calcareous soil. An isotopic exchange reaction occurs between the residual ^{59}Fe chelate and the soil iron. The isotopic exchange of ^{32}P between added fertilizer and soil phosphates has also been examined. Preliminary results on a soil low in phosphate suggest that considerable reliance may be placed on calculations of fertilizer phosphate uptake based on ^{32}P assays. Work with other types of soil is in progress.

Iron contamination of tomato foliage by dust was shown to occur in the glasshouse, and a suitable washing technique was devised. Radioassays on tomato grown in water culture and given iron labelled with ^{59}Fe showed that no loss of iron occurred from within the tissue during this washing. Experiments are in progress with radioactive isotopes to investigate the distribution of iron and molybdenum in dialysates of *Azotobacter* and *Neurospora* (see Nutrition of Micro-organisms, p. 43).

Work has continued on the physico-chemical aspects of spray application. The effect of a polyvinyl acetate latex supplement on the tenacity of cuprous oxide has been determined in field trials on daffodil (in collaboration with N.A.A.S., Starcross) and potato foliage (at Starcross and Long Ashton). The tenacity of this formulation, as determined chemically, did not equal that of a standard Bordeaux treatment. Work has been carried out on the effect of the nature and particle size of deposits of six copper fungicides upon their tenacities on artificial and leaf surfaces (p. 111). At the same level of deposit, tenacity appeared to be independent of the type of distribution and to be more dependent upon particle size. In work with " run-off " washes, different levels of fungicide deposit were obtained with different types and concentrations of wetting agent.

Work on the laboratory determination of effective particle size distribution of DDT dispersible powders has been completed.

Entomology.

Biology of insect pests.

Observations on the aphid species infesting apples in 1955 showed the Oat Apple aphid (*Rhopalosiphum insertum*) to be predominant at the beginning of the season ; infestation by *Aphis fabae*, *Macrosiphum euphorbiae*, *Aphis pomi* and *Eriosoma lanigerum* occurred later. Only the last caused severe infestation where control measures were not applied.

The biology of an unidentified sawfly (*Ametastegia* sp.), found boring into the cut ends of the leading shoots of one- and two-year-old apple trees planted in grass is under investigation (p. 120).

From further study of the emergence habits of the Button Top midge of basket willows it was evident that the modern practice of removing one-year-old rods, containing midge puparia, from the beds to a distant point for processing, was reducing infestation in the following season. Preliminary experiments in the control of the Willow Bean-gall sawfly attacking basket willows are described in the Willows Section (p. 38).

Bio-assays of insecticides and fungicides.

In laboratory assays of materials for the control of winter eggs of the Red Spider mite it was found that at 0·02 per cent. concentration both chlorbenside and chlorfensone gave a kill of not less than 97% of the eggs, provided they were applied just before egg hatch. The survival of 2–3 per cent. of eggs under favourable climatic conditions may be sufficient to cause a moderate late spider attack (p. 125).

Work on the contact activity of isosteres and analogues of DDT was completed. The replacement of the chlorine atoms of DDT by methyl or hydroxyl groups was found to cause a marked loss of activity against the locust, grain-weevil and cotton stainer (41).

Using DDT dusts and topical applications of DDT in arachis oil an investigation is being made of the effect of " pick-up " in the determination of toxicity by means of filter paper assays. Work was begun on the effect of insecticides on respiration, and further studies were made of their effect on the nervous system.

Conjoint work with the Chemistry Section on the systemic insecticide demeton-S is described on p. 30. Using a number of other systemic insecticides, including *bis*(2-fluorethoxy) methane, dimefox, dimeton, demeton and schradan, distribution through the plant was found to be best following root absorption. The very variable rates at which toxicity was translocated following leaf applications showed the need for an investigation of the physiological processes involved.

The DDT/*gamma*-BHC emulsion of high wetting properties described in the Report for 1953 has been in use commercially in 1955 as a substitute for winter washes, and has proved considerably cheaper. In experiments in which its application was delayed until the full pink bud stage of apples there was still complete control of aphid and no apparent interference with fruit set. The inclusion in the pink bud spray of 0·02% chlorbenside for the control of the winter eggs of the Red Spider mite (Report for 1954) did not impair its efficiency against aphids.

Further field experiments were made to determine the influence of time of application and of the inclusion of a wetter in spring washes. Using the Oat Apple aphid as test insect, it was found that BHC wettable powder applied at any time from the mouse-ear to the green-cluster stage gave a high control, both with and without wetter, when spraying ensured an appreciable deposit of fluid on all the buds. But for the joint control of aphis and sucker under commercial conditions, particularly where the trees are large, the inclusion of a wetter in the DDT/BHC emulsion has been shown to be very necessary.

Field trials on the effect of different spray application techniques on the control of apple and pear scab were continued in collaboration with the Plant Pathology Section. Small volume air-flow mist applications of dilute captan gave excellent control of pear scab on trees planted and managed to suit automatic methods. The same application method with the same material failed to control apple scab on large spreading trees unsuited to automatic spraying.

Distribution of spray materials.

For further study of the production of spray droplets (1954 Report) an experimental spinning disc is being built to obtain information on the effects of disc speed and design in relation to the physical properties of spray fluids.

The effects of surface-active agents incorporated as spray supplements are being explored by static and dynamic methods. The static methods include the measurement under the microscope of individual spray droplets after impaction on leaf and other surfaces and the determination of the degree of adsorption of the surface-active agents by leaf surfaces.

For dynamic studies a high speed photographic method is being developed to examine the impaction of spray droplets on solid surfaces and for measuring the contact angle and behaviour of the drops as they move over the surface. The retention of active material on the surfaces is being measured by chemical or biological means. Transparent impressions of plant surfaces obtained in cellulose acetate or gelatin (p. 121) are proving useful in this work.

Laboratory experiments were carried out on the influence of site of deposition of fungicides on development of blight on potato leaves and chocolate spot on bean leaves. In the study of the mode of action of fungicides uniformly applied as small discrete drops, the percentage cover has been related to the degree of control of *Botrytis fabae*. The percentage cover required with Burgundy mixtures for complete control varied with the supernatant copper content.

In field experiments, made in conjunction with the Chemistry of Insecticides Section, the distribution of spray materials on the target surface was determined by finding the amounts of DDT deposited on apple fruit buds at various stages of development. A copper fungicide was also used as an indicator for studying the effect of different techniques of application on the distribution of spray materials on apple foliage. Both percentage foliage area covered and the amount of spray material retained were shown to be greater with large volume than with small volume methods. Percentage cover on the under-surfaces of the leaves was greater than that on the top with large volume hand- and small volume mist-spraying, but less in the case of large volume automatic mast spraying. In experiments with potato foliage the percentage cover on the under surfaces was greater with small volume mist spraying than with small volume boom spraying. The addition of a wetting agent increased percentage cover on the undersurfaces of the foliage.

Solutions of copper sulphate of various strengths were used with succinate wetter to find the optimum concentration needed to control potato blight without damage to the foliage. In the absence of natural infection plants inoculated by spore cultures were used.

Experimental work on the control of Leaf Spot of bananas by copper fungicides in Jamacia has been completed. The variety Lacatan is almost entirely subject to ascospore infection, which is confined to the newly-opened top leaf for a very short period. Infection can be controlled by copper fungicides only if they are uniformly distributed on the underside of the leaf : a new technique of spraying was devised and will be put into practice throughout the Island in 1956. Analytical work on the retention and distribution of the copper fungicides has been carried out in collaboration with the Insecticides and Fungicides Section.

Spray Machinery.

The design of experimental equipment for the Colonial Office has entailed visits to Germany and Jamaica.

A tractor drawn and driven airflow " mist blower " was designed and made for experimental work on coconut palms in Zanzibar. This machine, with a delivery of 8,000 cu. ft. of air per minute and small droplet sizes, is likely to have many uses for the application of concentrated spray materials to tropical crops (p. 151).

The difficulty of observing the distribution of spray droplets on tall trees has been reduced by adapting lifting and ladder equipment. A high-lift platform hydraulically operated from a tractor has proved satisfactory on apples and pears (p. 156). A trailer-mounted self-supporting ladder has been used for examining the tops of 70 ft. high coconut palms (p. 157).

Small lightweight power machines of the type described in the Report for 1954 were made for use with coconuts in the Solomon Islands, citrus in Kenya and bananas in Jamaica.

Plant Pathology.

Field Experiments.

In investigating sources of infection for *Gloeosporium* rot of apples, the spore dissemination of *G. perennans* was followed, using cankers resulting from inoculations on branches of Cox's Orange Pippin. Spores were washed off by rain throughout the summer, the maximum monthly spore output being in September. In co-operative experiments with the Department of Scientific and Industrial Research on orchard control of the disease, Allington apples were sprayed in September 1954 with 0·1% captan and cold-stored at the Ditton Laboratory : these in January 1955 showed approximately half the *Gloeosporium* infection found in the controls. Late-summer spraying trials on Allingtons were repeated at Long Ashton in 1955.

In joint experiments with the Entomology Section, six small-volume (100 gal./acre) sprayings of 0·1% captan, 2% and 4% lime sulphur failed to give satisfactory scab control when applied to large, 30-year-old Cox and Worcester trees. On a plot of pears, grown on the " hedgerow " system and so better suited for small-volume spraying, effective scab control on the variety William's was obtained by six sprayings at 80 gal./acre, using either 1% lime sulphur or 0·1% captan.

For the second year of a trial on Cox, comparing lime sulphur and captan as post-blossom sprays (following lime sulphur pre-blossom), both treatments

gave excellent scab control. Yields were substantially higher on the captan-sprayed blocks and russeting lower. Less leaf infection by apple mildew occurred on the lime sulphur-sprayed trees, but overwintering bud infection by mildew remains low throughout the plot.

Results of the assays of processed strawberries from the 1954 spraying trials have been published (31). Thiram spray residues induced a marked taint in canned fruit; captan a slight taint. Jams were not affected. These preliminary experiments were followed by a joint Long Ashton-East Malling-N.A.A.S. trial of *Botrytis* control measures on a plot of Royal Sovereign strawberries at the Luddington Experimental Horticulture Station in 1955. The trial comprised eight replicates of seven fungicide treatments and a control. In a season unfavourable to *Botrytis* attack the results showed only that the unsprayed and the dusted plots had more *Botrytis* than any of the sprayed plots. Fruit from all treatments was processed at Long Ashton by canning, quick-freezing and jam-making, and the products were examined after storage.

The gooseberry mildew trial on the sulphur-sensitive variety Leveller was continued at Luddington. There was a light attack of mildew on the bushes sprayed with zineb : none occurred on the plots sprayed with Karathane or with washing soda plus soap. No spray injury was noted in 1955.

In phytotoxicity trials with antibiotics, penicillin and streptomycin sulphate, at 500 p.p.m., caused no damage to leaves and fruit of Cox apple.

Laboratory Experiments.

Trials with twenty apple varieties showed that resistance to spore infection by the brown rot fungus *Sclerotinia fructigena* was significantly correlated with the rate of browning of the cut fruit surface. The brown oxidised tannins were not themselves fungicidal. The production of an exocellular acetylesterase by *Sclerotinia laxa* has been demonstrated and its activity examined (11).

Fungicide tests on overwintered black currant leaves infected with the leafspot fungus *Pseudopeziza ribis* showed that sporulation was inhibited by the sodium salt of DNC, or by sodium pentachlorphenate or sodium arsenite (14).

Experiments on the range of action of micro-deposits of captan on pear leaves demonstrated that pear scab spores in a water film on the leaf surface were prevented from germinating in an area of 4 sq. mm. around the deposit from a 0·0005 ml. drop of captan of normal spray strength. A corresponding deposit from captan of eight times normal strength protected an area of 9·6 sq. mm. (p. 127).

Cider and Fruit Juices.

Cider and Perry.

Vintage trials of cider and perry varieties have been continued and the trials of cider apples have been extended to include some lesser known varieties that may be worthy of inclusion in a revised list of Varieties Recommended for Planting. This work has been undertaken in collaboration with the N.A.A.S. and the Station Orcharding Committees. In these trials the adoption of a standard method of fermentation combined with sterile bottling or pasteurisation has been found to give a better assessment of the vintage quality of fruit samples and to simplify experimental procedure.

Work on juice and cider composition now includes an examination of nitrogenous components, organic acids and carbohydrates. The study of amino-acids continues : it has been found that the extent of yeast autolysis has a great influence upon the nitrogen content of a cider. The products of yeast autolysis include a wide range of amino-acids and peptides and encourage subsequent deterioration of the cider due to the growth of bacteria. An unknown amino-acid is a main component of some pear juices and perries and is being studied in detail.

Earlier work on the organic acids of apple and pear juices and their ciders and perries is being published and more detailed work is now in progress. Shikimic acid, an intermediate in the reduction of quinic to dihydroshikimic acid, has recently been tentatively identified in apple juice. The changes in organic acids produced by the lactic acid bacteria in ciders are being followed by the use of pure cultures as growing cells or as washed or homogenised cell suspensions. The practical cider-making aspects of this work are discussed at p. 159 of this Report.

The carbohydrates not fermented by yeasts have been found to consist of a range of sugars and related substances. They include xylose, galactose, ribose, rhamnose and oligo-saccharides : hexitols together with meso-inositol can amount to 0·4–0·8% by weight in some ciders.

Improved methods using selective media for the isolation of yeasts and bacteria from mixed populations have been described (1, 2). A further publication (3) has described a technique for simplifying the problem of making multiple routine inoculations of micro-organisms. Attention is now centred mainly on the classification of yeasts and lactic acid bacteria isolated from juices and ciders and on their nutrition and metabolism. Many of the yeasts isolated are capable of producing a wide range of amino-acids from ammonia as sole source of nitrogen and of assimilating organic acids or producing them from carbohydrate. The metabolic pattern of the lactic acid bacteria varies according to species and to pH. The significance of these changes in relation to cider-making practice is being studied on both the laboratory and factory scale.

In commercial practice sulphiting low-acid juices has been found to have many advantages : the main fermenting yeasts of the juice are encouraged and non-fermenting yeasts and bacteria are suppressed. Sulphiting leads to a more rapid and effectively pure-culture fermentation by the fermenting yeast naturally present (see p. 169).

Unfermented juice products.

Interest in the metallic components occurring naturally in apples and apple juices has been confined mainly to copper. The total amount of copper in an apple increases during the growing season, but the concentration in the tissues decreases. It is not related to the concentration of the copper-containing enzyme polyphenoloxidase. The ionic state of copper in apple juices has been studied in detail and the degree of complexing with other juice constituents such as malic acid, amino-acids and polyphenols has been investigated. In juices polyphenol-copper complexes appear to form an oxidation-reduction system and to play an important part in determining the stability of bottled apple juices. The formation of storage deposits in such juices is directly related to their copper content.

Factors influencing the stability of concentrated apple and soft fruit juices continue to be investigated. Work on the effect of storage temperature upon quality deterioration in black currant, raspberry and strawberry concentrates has been published (40). The factors governing the stability of unconcentrated juices stored under sterile conditions are being further studied.

Domestic Food Preservation.

Investigations of the survival of heat-resistant bacteria in bottled or canned meat and poultry, processed with domestic equipment, have been continued. The times of processing required have been studied for different sized containers, using pork, sausages, brawn and joints of chicken.

The present Report contains an account of some of the factors of importance when deep-freezing pork products (p. 188). Wrapping material must not only prevent loss of moisture, with consequent drying of the surfaces, but must also exclude air, to delay the onset of rancidity. The cut surfaces of cured meats must be kept to a minimum and salt should not be added to sausages before freezing.

The deep-freezing of chickens for periods up to 18 months has given good results, both with whole birds drawn and trussed ready for cooking and with joints packed in suitable containers : after thawing, the chicken could be cooked by any of the usual methods. Contrary to the information generally given in the literature, killing by dislocating the neck was found satisfactory, provided that the heads were kept down during plucking and cooling.

Classes were held in the spring of 1955 for selected County Instructresses in Rural Domestic Economy, Ministry of Education representatives, and lecturers from Domestic Science Training Colleges, to demonstrate the progress made so far in the preservation of meat and poultry.

Comparisons have been made of the qualities of 14 raspberry and 12 strawberry varieties, both as fresh fruit and after preserving by bottling and deep-freezing. The results obtained have been compared with those of previous years (p. 182). One of the most outstanding points noted was the great superiority in both colour and flavour of bottled fruit stored in the cold (38°F.) over that stored at room temperature. Previously all the fruit bottled for variety trials had been stored at room temperature.

The raspberries were made into jam to examine their tendency to develop " blindness," a fault characterised by a dullness in the jam and translucency in the seeds, previously noticed during the storage of jams made from some of the varieties being tested.

Ten varieties of pears have been tested for the minimum temperature required to inactivate the enzyme systems responsible for browning of the cut fruit. Previously 190°F. had seemed sufficiently high to prevent this discoloration occurring in bottled pears, but it seems that some varieties may require even higher temperatures. The pear varieties were assessed for their bottling qualities but were not frozen, as previous trials had shown pears to be unsatisfactory when frozen by the usual methods.

Tasting trials have been continued with strawberries sprayed for the control of *Botrytis cinerea* (see Pathology Section, p. 35) : fresh, canned and deep-frozen fruits and jams are being examined. In 1954 thiram was found to cause marked taint in canned fruit, but no taint was detectable in fresh

fruit or jam ; slight taint was also detected in canned fruits that had been sprayed with captan. In the 1955 trials, not yet completed, it seems likely that the presence of Strawberry mildew on the samples will preclude the obtaining of reliable results.

No taint was detected in canned fruit, frozen fruit, jams and syrups prepared from black currants sprayed with a high concentration of lindane.

Trials of the preserving qualities of 32 different varieties of vegetables have been made and the amount of waste during the preparation of each variety, when picked slightly immature and when mature, has been noted. The results for those varieties tested for three years are given in this Report (p. 178).

Bacillus thermoacidurans, an organism frequently causing spoilage in tomato products, has been further tested for its resistance to boiling for different lengths of time in tomato medium with the pH values adjusted to the range normally found in tomatoes. It has been found to be much less heat-resistant in the more acid tomatoes.

Willows.

Variety Collection.

The examination of varietal characteristics likely to assist in the classification of basket willows has been continued. Particular attention has been paid to 61 varieties received from Scotland in 1954, of which 12 appear to be synonomous with existing members of the Station variety collection.

Experimental Work.

In further studies of the root pattern of basket willows in relation to possible damage by mechanical cultivation, measurements were made for the second year on a plot planted in 1954. It was found that the likelihood of injury in this plot was not great : only 10·8% of the roots occurred in the surface 3 inches ; 40·0% occurred at a depth of 3–6″.

In collaboration with the Entomology Section, preliminary observations were made of the toxicity of soil applications of BHC, dieldrin, and tar oil emulsion to the larval cocoons of the Willow Gall sawfly, *Pontania gallicola*, a pest that was unusually prevalent in Somerset in 1954. None of the treatments gave any significant control of the insect.

Data obtained during a survey of the Somerset basket willow industry show that the cost of establishment of willow beds is approximately £260 per acre, of which £160 represents the cost of the land. The annual cost of management, including depreciation on establishment cost, is between £50 and £70 per acre, while the average value of the crop is about £100 per acre.

Advisory Work.

There were 173 requests for information during the year. 30 visits to growers were made as below :—

	South West.	*Home Counties.*	*East Anglia.*	*Midlands.*	*North.*	*Foreign.*
Number of enquiries	74	48	22	8	13	8
Number of visits	14	7	6	–	3	–

Three-quarters of the enquiries were concerned with basket willows and one quarter with bat willows (*Salix alba* var. Caerulea).

Requests for planting material included four from research and educational centres at which it will now be possible to study the growth of a large range of varieties in widely different climatic conditions. Varieties sent to Scotland (Forestry Commission) have again made poorer growth than local varieties. The more vigorous of these Scottish varieties are being planted in N.E. Scotland, where there is a great shortage of basket willows.

Varieties of *Salix triandra* were sent to the Rural Industries Bureau (London) for an investigation into the use of steam heating, as opposed to boiling, in the peeling process. The extraction of salicin from the peelings was found to be uneconomic.

The acute shortage of basket willows in 1954 has stressed the need for a reliable estimate of national production. It is hoped that data collected in 1955 to show the distribution and age of the varieties grown in Somerset, the most important producing area in Great Britain, will prove helpful.

The possibility of using willows for paper pulp production has been studied at the request of the Forestry Commission. Waste from the cricket bat willow has been found suitable, and there is a possibility of utilizing the crack willow (*Salix fragilis*). The use of basket willows of the required size has proved uneconomic.

Statistics.

Experimental Design and Analysis.

A layout has been prepared for a long-term experiment in pear nutrition and spraying on the Station (plot 7). A design has also been provided for the long-term recording, on a site in Herefordshire, of apomictic stocks at present undergoing nursery trials at Long Ashton. Considerable time has been devoted to a study of the problems arising in the planning and conduct of cider and perry varietal trials, in preparation for new trials at Long Ashton and elsewhere.

Advice has been given to members of all sections of the Station staff in the planning of experiments and the interpretation of results. The necessary analyses have taken a considerable amount of time, during which attention has also been paid to improving calculation techniques. A feature of almost all sampling carried out at Long Ashton in 1955 has been its prior design along valid statistical lines. Established principles in the abstraction of samples from large populations have been applied in a wide variety of problems.

In the organisation of a tasting panel for examining several samples of fruit each subjected to different treatment, it is necessary that each member of the panel shall taste a limited number of samples. Further, it is desirable that all tasters should be given a " control " treatment as one of their samples. An arrangement, with its corresponding statistical analysis, has been developed which uses a modification of the standard " balanced incomplete block " design.

Biometric Studies.

The investigation begun in 1954 on the variation in environmental conditions in the large pot-culture greenhouse has been continued ; the information

obtained will enable full advantage to be taken of statistical principles in designing experimental work. Experience of the technique of such investigations will also prove useful in examinations of the new range of greenhouses.

A rapid method of assessing leaf spot in black currants is described in this Report (p. 196). The method enables the labour of field observation to be reduced greatly, while the results obtained are comparable in precision with those obtained by previous methods.

Measurements of shoot length on several varieties of black currants have been made in 1955, and will be used to study their correlation with crop weight in 1956. Other measurements of both length and weight of pruned shoots of several varieties have been taken for correlation studies.

Data available for the black currant varieties Cotswold Cross and Mendip Cross grown at Long Ashton have been examined by regression methods for relations between crop weight in a season and leaf nitrogen content in that and the previous season ; although the crop weights were shown to be highly correlated with leaf nitrogen content in both the same and the previous season, no clear indication of the magnitude of the nitrogen effects on crop could be found.

AGRICULTURAL RESEARCH COUNCIL UNIT OF PLANT NUTRITION (MICRONUTRIENTS).

Plant Physiology.

Effects of molybdenum on growth or metabolism. Investigations are being made into factors which affect the apparent activity of nitrate reductase, acid phosphatase and enzymes which oxidise ascorbic acid when extracted from tissues of cauliflowers grown in sand culture. Plants grown with nitrate or nitrite show considerable nitrate reductase activity whilst those grown with ammonium sulphate show much less but not always negligible activity. Molybdenum deficiency levels of the same order as those causing whiptail symptoms produce large decreases in total activity in plants grown with nitrate or nitrite and also small decreases in plants grown with ammonium sulphate. Plants grown with 10–20 p.p.m. molybdenum show apparently normal nitrate reductase activity (p. 202).

The apparent activity of nitrate reductase varies greatly in different parts of the plant and rises to a maximum in mature leaves just prior to senescence. The distribution and relative amounts of extractable leaf protein differ from those of nitrate reductase but correspond with acid phosphatase activity.

Vacuum infiltration of molybdenum deficient leaves by molybdate solutions has not produced consistent results for nitrate reductase so far in cauliflower plants grown with nitrite, and only small increase in activity was observed after 24 hours. Acid phosphatase activity, which is inhibited by molybdate in some preparations, e.g. tomato, was not appreciably affected in these tests.

Exclusion of light or air greatly decreases apparent nitrate reductase activity without a corresponding decrease in total extractable protein. The relation of nitrate reductase activity to the distribution of nitrite reductase and possible sources of interference by ascorbic acid are being investigated.

Ascorbic acid oxidase located in, or on, the cell walls appears to account for a great part of the extractable ascorbic acid oxidative activity of the cell.

Activity is greater in young than in old leaves of mature plants but no consistent effect of nitrogen or molybdenum supply has been detected. Soluble enzymes, extracted by centrifuging frozen tissues after thawing, showed a greater oxidative activity from molybdenum deficient than from normal tissues of young plants. Although cytochrome oxidase and polyphenol oxidase, as revealed by oxidation of ascorbic acid, could not be detected in suitable preparations, further evidence will be sought on the effects of molybdenum supply on enzymes which may be involved in maintaining the steady state of ascorbic acid in tissues (p. 211).

The concentrations of adenine in leaves were estimated, as earlier investigations showed that 5-adenylic acid accumulated in molybdenum deficient cauliflower leaves and adenine has also been suggested as a possible growth factor in certain tissue cultures. Molybdenum deficiency had no effect on adenine content except in plants grown with nitrate, where it was decreased.

Enzymes in nitrogen metabolism: *aldehyde oxidase.* This enzyme is of interest as it catalyses the reduction of nitrate anaerobically in the presence of acetaldehyde without the addition of pyridine nucleotides and differs from nitrate reductase in other respects. The enzyme may therefore provide an alternative mechanism to nitrate reductase for nitrate reduction in some tissues. It is being fractionated from potato tubers and the nature of its cofactor requirements, including metals if any, and its distribution in plants are under investigation.

Essentiality of elements for higher plants. The investigations begun in 1951 in sand culture and renewed in water culture in 1954 were continued. In spite of attempts to exclude dust and of extensive purification of reagents and culture equipment, no response was observed to the supply of elements, including chlorine, additional to those generally recognized as essential. Spectrographic examination, by the Macaulay Institute, of foliage and roots produced in 1954 revealed unexpectedly high contents of several elements. Direct contamination of the foliage may have occurred and a conditioned air supply is considered necessary for the extension of this work.

Tissue cultures. In preliminary work with tissue cultures, tomato root clones were isolated from adventitious roots from sterilised stem segments and their suitability as material for physiological work is being examined.

Analytical: *ascorbic acid and ascorbic acid oxidase activity.* The light absorption of ascorbic acid at 265 mμ in aqueous solution at pH values above 5·5 has been employed as the basis for a spectrophotometric method for the enzymatic estimation of 5–50 μg ascorbic acid in the presence of pigments in neutralised metaphosphoric acid extracts from a few milligrams of tissue. Good recoveries of ascorbic acid added to plant extracts have been obtained and the method is suitable for routine use. Investigations are in progress to extend the method to dehydroascorbic acid. The spectrophotometric method is also suitable for the assay of enzymes that oxidise ascorbic acid in tissue preparations, and optical density changes of 0·1 per minute are produced by 50 μl of cell sap.

Incidental results show that whereas ascorbic acid is stable in fresh detached leaf fragments for 24 hours at room temperature, there may be a relatively rapid loss after a few hours at —10°C.

Culture methods. The use of ammonium sulphate as a source of nitrogen in water culture and the growth of cauliflower in water culture are technical

problems involved in future growth analysis investigations using this method of culture.

The toxicity of ammonium compounds is mainly eliminated by suspending calcium carbonate in the culture solution, and a method to achieve this is being developed. Cauliflower plants do not grow well when the roots are fully immersed in the culture solution even when aerated, but satisfactory growth has been obtained when the root system is only partly immersed and the exposed parts are kept in a saturated environment. Atomised culture systems have proved unsatisfactory for this purpose. The development of water culture methods for growing cauliflowers from seed with ammonium sulphate is still under investigation.

Nutrition of Micro-organisms.

Role of trace metals in enzyme systems of micro-organisms.

Molybdenum in nitrate reductase from Neurospora crassa. Molybdenum has been identified as a constituent of nitrate reductase in *Neurospora crassa*, *Aspergillus niger*, *Escherschia coli* and in higher plants (33, 34). The micronutrient undergoes oxidation-reduction changes during nitrate reductase action in the following sequence :—

Reduced di- or tri-phosphopyridine nucleotide → Flavine adenine dinucleotide or flavine mononucleotide → molybdenum → nitrate ion

It has now been established that during the enzymatic reduction of nitrate the valency change undergone by molybdenum involves Mo^6 and Mo^3.

Sodium molybdate reduced with sodium dithionite, which acts as an electron donor for the enzymatic reduction of nitrate, was shown to contain Mo^5 as the most reduced valence state of the micronutrient. Further, Mo^5 prepared free from molybdenum in the other valence states acts as an effective donor for the enzymatic reduction of nitrate but Mo^6 is without effect. Mo^3, which will reduce nitrate non-enzymatically, is unlikely to be important in plant metabolism as it is readily oxidised to Mo^5 by Mo^6 or by other metals in the oxidised state, which are invariably present in plant cells. Mo^4 is only stable in strong ethanol solution and it dismutes readily to form Mo^5 and Mo^3 in the presence of a trace of water and is therefore of little importance in nitrate reduction (35).

Nitrate reductase, purified 50 fold by ammonium sulphate fractionation, gel and carbon adsorption techniques, appears to require phosphate for maximal activity. Thus the oxidation-reduction of molybdenum probably occurs through a phospho-molybdate complex. The —SH groups on the enzyme may be important in binding the phospho-molybdate to the apoenzyme.

Molybdenum in relation to Iron Enzymes. Catalase and peroxidase are much reduced in felts of *N. crassa* that are deficient in molybdenum. Work is in progress to determine the role of molybdenum in the utilization of hydrogen peroxide in fungi.

Succinic acid and Riboflavin mutants of Neurospora crassa. A mutant of *N. crassa* requires riboflavin when grown at 30° but there is no requirement

for the vitamin at 25°. A zinc deficiency is more readily produced when the mutant is grown at 30° which may indicate that zinc is involved in the synthesis of flavoproteins from the riboflavin supplied.

The following enzyme changes were observed when the mutant was grown at 30° with a limited amount of riboflavin : significant increases in peroxidase, cytochrome C oxidase, and a decrease in catalase, cytochrome C reductase, diaphorase, nitrate and nitrite reductase, as compared with the mutant receiving an optimal quantity of the vitamin. The incorporation of riboflavin into flavoprotein enzymes was studied.

The iron and molybdenum requirements of the succinic acid mutant of *N. crassa* were less than in the wild type. A deficiency in succinate resulted in an increase in catalase, peroxidase, cytochrome C oxidase, succinic dehydrogenase, fumarase and nitrate reductase, and a decrease in cytochrome C reductase and fructokinase.

Molybdenum, Vanadium and Iron in relation to growth and metabolism of Azotobacter. In *Azotobacter chroococcum* O strain, strains 8003, 8004, 8514, 8515, and in *A. vinelandii*, iron deficiency was more readily produced than molybdenum deficiency. Strains 8514 and 8515 required less iron or molybdenum than strain 8003. Vanadium or tungsten replaced molybdenum to the extent of 80% or 30–50% respectively for growth.

Hydrogenase, the enzyme which catalyses the oxidation of molecular hydrogen with subsequent reduction of cytochrome in *Azotobacter* was found to be reduced by one-fifth and one-half of normal values in bacteria deficient in iron and molybdenum respectively. The enzyme is associated with cell particles and is therefore difficult to purify. The mechanism of hydrogenase action is probably :—

$$H_2 \longrightarrow \begin{matrix}\text{flavine adenine}\\ \text{dinucleotide}\end{matrix} \longrightarrow (\text{Fe.Mo}) \longrightarrow \text{Cytochrome.}$$

Work is continuing to determine the role of the two metals in the electron transport sequence and to study the relation of hydrogenase to nitrogen fixation in *Azotobacter, Clostridia* and *Rhodosporillium.*

Iron and molybdenum were found to be associated with cell particles in *Azotobacter*, as is hydrogenase (36, 37). In collaboration with the Physical Chemistry Section, the bacteria were grown in pure cultures, some containing ^{59}Fe and others ^{99}Mo. Homogenates of the bacteria were prepared and these were dialysed against various buffered solutions, including chelating agents. It was found that molybdenum was released readily from the particles by dialysing against phosphate buffers whereas iron was more tightly bound to the proteins and was removed only by strong chelating agents. About 50–60% of the total molybdenum was removed by dialysis against a phosphate buffer (pH 7·5) but this treatment had little effect on iron. Versene removed 30% of the iron but was without effect on molybdenum.

Similar experiments were made with *Neurospora crassa.*

Mo^6, Mo^5 and Mo^3 solutions were supplied to *Azotobacter* grown in aerated cultures. Molybdenum uptake and nitrogen fixation were greatly enhanced when the lower valence states, Mo^5 and Mo^3, were supplied. Thus the first stages of N fixation are probably reductive, involving the lower valence states of Mo.

Effect of fungicides on enzymes in Sclerotinia laxa. In collaboration with the Pathology and Chemistry of Fungicides Sections, felts of *Sclerotinia laxa* treated with captan, copper sulphate, lime-sulphur and *o*-hydroxy-diphenyl were examined for enzyme changes. Preliminary results indicate that the fungicides affect differentially the iron, flavine and dehydrogenase enzymes.

Iron status of plants.

Iron which may be present as a serious contaminant on the leaves of tomato grown in glasshouse may be removed readily by washing the plant for 30 sec. in water containing 0·3% detergent. In collaboration with the Physical Chemistry Section it was shown that there is no loss of iron from plant tissues during the washing period as no radioactive iron could be detected in wash solutions of plants containing ^{59}Fe. There is no evidence that Fe is either adsorbed on silica during acid digestion of a variety of dried plant material or is present in soluble or insoluble complexes in the digest solutions.

Inorganic Chemistry.

Work on the separation of molybdenum valencies has been continued, and investigations have also been made of the valence states of vanadium and cobalt.

Molybdenum. The chromatographic separation of the hexa- and pentavalent thiocyanates on paper was shown to be quantitative, but the separation of the oxinates was found not to be so. The valence state hitherto denoted as " less than 5 " was shown to be 3 ; its separation from zinc and pentavalent molybdenum was carried out on a column of cellulose in order to prepare pure solutions for use in biological experiments (35) and to study the reducing properties of the trivalent state.

Separations of hexa- and pentavalent molybdenum as phenol derivatives of the chlorides were found to be inferior to those already obtained using the thiocyanates and oxinates.

Vanadium. Using paper chromatography, separations were effected between penta- and quadrivalent, and between quadri- and trivalent vanadium, and it was shown that neither of the separations was quantitative. Solutions of the trivalent state (used in biological experiments, and in an investigation of the reducing powers of the valency) were prepared by zinc-acid reduction of quadrivalent vanadium solutions followed by the removal of zinc on a cellulose column. Vanadium solutions and fractions were estimated colorimetrically by the extraction of the pentavalent state as oxinate into chloroform.

Cobalt. A separation of di- and trivalent cobalt as acetates was carried out by paper chromatography. The separation was not quantitative owing to the susceptibility of the trivalent state to reduction on the paper strip. Cobalt fractions were estimated colorimetrically as the blue cobaltous thiocyanate complex in acetone.

Organic Chemistry.

A substance believed to be *p*-coumaryl quinic acid (1954 Report) has been found in the leaf and fruit of the apple and pear. More precise examination of the material by paper chromatography resolved it into two distinct compounds which seem to be analogous to chlorogenic and neochlorogenic acids in the caffeyl quinic acid series. Substances chromatographically identical with these

p-coumaryl quinic acids have been observed in the leaf and fruit of many other Pomoidae (52).

In the examination of the *p*-coumaryl quinic acids on paper chromatograms the formation of double spots in a 2% aqueous acetic acid solvent by substances apparently homogeneous in other solvents was observed. Similar behaviour had been previously noted with chlorogenic and caffeic acids. It has been shown that the two spots are due to the presence of both *cis*- and *trans*- isomers which are resolved only in the aqueous solvent (50). An increase in the strength of the *cis*-spot was obtained in all cases after irradiation of the solutions with ultra-violet light and it was found possible to separate the pure individual isomers. On exposure to daylight the individual forms revert to an equilibrium mixture.

Small quantities of one of the leuco-anthocyanins of pear bark have been separated on a cellulose column but so far no crystalline derivatives have been obtained. With warm aqueous acid it yields epicatechin and complex bodies, and appears to be identical with the leuco-anthocyanin L1 separated from cacao beans by Forsyth.

Examination of the minor phenolics of the apple tree continues. In certain apple species a new substance, believed to be 3-hydroxy-phloridzin, has been found which partly replaces phloridzin in the typical phenolic pattern of the apple tree for these species.

Bark extracts from a number of perry pear varieties showing varying degrees of incompatibility with quince rootstocks have been examined. Although clear differences in the phenolic patterns were often visible there was no obvious relationship between these and the observations on degree of compatibility.

Study of the organic constituents of the tomato leaf continues. Some interesting variations in the acidic and carbohydrate fractions with change in the levels of major nutrients have been noted.

STEMBUILDERS FOR CIDER TREES: NURSERY TRIALS.

By A. I. CAMPBELL.

Introduction.

Since most cider orchards are grazed, a tree with a trunk of 6 ft. or 6 ft. 6 in. is necessary, and the method of raising such trees is very different from that used for dessert apple or pear trees, where a trunk of only 2 ft. 6 in. is required. Many of the most popular cider varieties are not vigorous enough to produce a standard tree without at least one or possibly two extra years in the nursery. Varieties such as Dabinett or Médaille d'Or may never be wholly satisfactory, and even after the additional period will produce small weak trees incapable of carrying heavy crops. Some varieties may be susceptible to apple canker or similar stem diseases and others may persistently grow away from the vertical. It is, therefore, usually necessary to use an intermediate stem, referred to as a stembuilder to distinguish it from the short length of stem used to overcome incompatibility between rootstock and scion.

It has been customary in Herefordshire to use another strong cider variety as a stembuilder and to bud it on the rootstock a few inches from ground level. The stem is then " run up " in two years and grafted with the permanent variety at 6 ft. 6 in. with a single scion ; the branches arising from the scion are then trained to form the framework of the tree. Fig. 1 shows a tree two years after grafting. The variety used almost exclusively in Herefordshire is Bulmer's Norman, a very vigorous grower that can easily reach grafting height in two years.

Sometimes the stem is grafted at 5 ft. and the top bud of the scion is " run up " another 12 in. or so before the head is formed. This method tends to reduce the chance of rogue shoots of the stembuilder growing out among the branches of the head, but the union part-way up the trunk tends to look unsightly.

In Somerset the variety Morgan Sweet is commonly used as a stembuilder. The method of tree production is somewhat different from that in Hereford. The stem is run up for two or three seasons and headed back at approximately 6 ft. 6 in. The head is then formed of the stem variety and the top grafting is done on the lateral branches. Fig. 2 shows such a tree two years after grafting. The grafting is sometimes carried out in the nursery, where the trees can be watched carefully for the first year and all shoots arising from the stem can be rubbed out. This method minimizes the risk of shoots being left in the head, but it is obvious that the greater the number of grafts the greater is the risk of rogue branches, so that the single scion method used in Herefordshire is somewhat better from this point of view.

Some farmers prefer to graft the tree with the selected variety after it has been planted in the orchard for one or two seasons. This method has the advantage of producing a larger tree in a shorter time, and of allowing the tree to become well established before grafting. Where gaps occur in the orchard this seems to be the most satisfactory way to treat replacements.

Very little is known at present of the interaction between the rootstock, stembuilder and tree head. It is evident from the work of Hatton and others (1) that the rootstock affects the scion variety and *vice versa*. Swarbrick (2) showed that a 6 in. piece of stem inserted between the scion and rootstock also had a marked influence on the growth and behaviour of the scion varieties. The trees that Swarbrick used in his experiments have again been examined after twenty years, and the influence of the short piece of stem is still very marked : in all cases the effect has been to reduce the size of the trees, probably because the piece inserted was of a weaker variety

It is well known that the vigour and shape of the tree head can be altered by the various rootstocks and it has been found that the effect on the scion variety of a stembuilder 6 ft. in length may be as great as or even greater than that of some of the rootstocks. The interaction between root, stem and scion variety is different for every combination that has been examined but, in general, if the stem variety is more vigorous than the scion variety, as is usual, then the resulting tree is larger than it would have been had no stem variety been used. The reverse also holds : if a weak stem piece is inserted or a weak variety is head-worked, the resulting tree will be smaller.

Many varieties have been tested at various times as stembuilders, and Barker (3) in 1937 mentions Bulmer's Norman, Bramley's Seedling, Sweet Alford and White Alphington as being under trial and giving promising results. These varieties have recently been examined and Bulmer's Norman seems to be generally the most successful : it tends, however, to make a weak crotch and if the Somerest method of tree-building is used the trees are liable to split in later years. Trees of Ellis Bitter worked on Bulmer's Norman stems are larger than similar trees with a stem of White Alphington but, on the other hand, trees of Médaille d'Or are of the same size on both stems.

Experimental.

Nursery Trial of Six Stembuilders

In 1951 six varieties, Bulmer's Norman, Morgan Sweet, Sweet Coppin, Yarlington Mill, Tremlett's Bitter and Crab C, all known to produce strong straight stems in the nursery, were included at Long Ashton in a further stembuilder trial in which both seedling cider (Knotted Kernel) and clonal (Crab C) rootstocks were used.

240 Crab C rootstocks were used, divided into 4 blocks, each containing 10 trees of each of the 6 stem varieties arranged in random order. There were only 120 seedling rootstocks, giving two blocks of trees similarly arranged.

The rootstocks were budded in August 1952 at 4 in. from ground level. In April 1953 the Crab C rootstocks to be " run up " as stems were cut to 4 in. at the same time as the budded rootstocks, in order that all varieties should start from the same height. There were only a few bud failures, distributed fairly evenly amongst the varieties ; these were grafted in the following spring. At the end of the first season the grafted trees were of normal appearance.

In the second season all the " feathers " and laterals arising from the main stem were pruned back at regular intervals to 4 or 5 in. Yarlington Mill and Sweet Coppin did not produce many feathers and this may account for the more slender stems made by these varieties.

During 1953 and 1954 monthly records were made of their height and diameter and of the susceptibility of the six varieties to various diseases, mainly apple canker, scab and mildew.

Results.

Fig. 4 shows the mean heights measured monthly from the top of the rootstock to the tip of the scion variety. The diameters were measured during both dormant seasons at a point 12 in. above the union.

It will be seen that the six varieties made maximum growth at different times. Bulmer's Norman, for example, grew extremely rapidly during the months of May, June and July, and then almost stopped, whereas other varieties had a much more even growth rate.

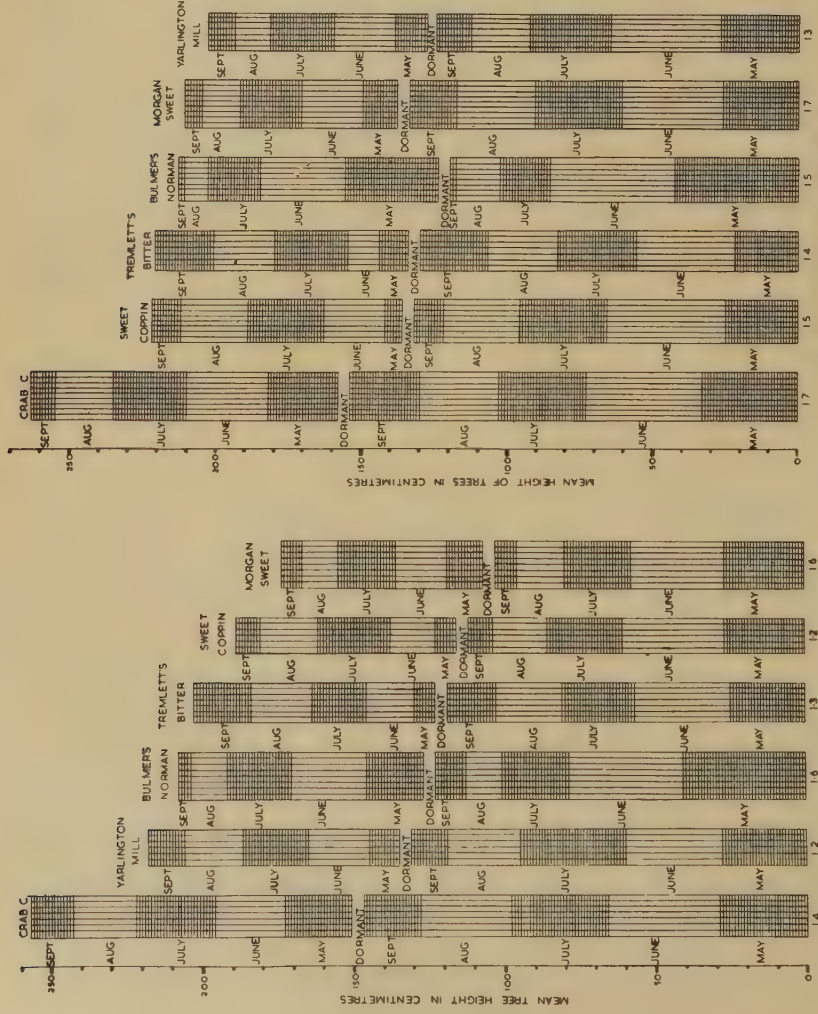


Fig. 4. Mean heights, and diameters after two seasons, of six stembuilders.
(a) Crab C rootstocks. (b) Seedling rootstocks.

Fig. 1. Crab standard two years after grafting; arrow indicates position of union.

Fig. 2. Crab standard two years after grafting on laterals formed by stembuilder; arrows indicate position of unions.

Fig. 3. Stembuilders in nursery two years after budding. Note vigour of Crab C compared with Bramley's Seedling.

Temperature and sunshine were a little above normal in 1953 and rainfall was also a little above average during the summer months. 1954 was by contrast a dull, wet summer, with temperatures well below average. Fig. 4 shows the growth of the trees during the two years; all six varieties made much less growth in the second year, but the differences between them remained unaltered. Fig. 3 shows the trees at the end of 1954; it can be seen that the trees of Crab C are much more vigorous than Bulmer's Norman.

The results show that in the two years the trees of Crab C made much longer stems than any of the five cider varieties, both when grown on seedling rootstocks and when grown on their own roots. Their stems were completely satisfactory in the nursery; their diameter was as great as those of Morgan Sweet on seedling rootstocks and greater than those of the other varieties. Their diameter was, however, less than those of Morgan Sweet and Bulmer's Norman when these varieties were worked on Crab C rootstocks.

Bulmer's Norman produced thick stems, especially when worked on Crab C, but they seemed very susceptible to apple canker (*Nectria galligena*) at Long Ashton. The plot in which the trees were grown is adjacent to an old orchard in which canker is fairly widespread, and the land lies rather wet in the winter. Morgan Sweet and Tremlett's Bitter were also infected by apple canker to a much greater extent than the other varieties.

Many older trees in which Bulmer's Norman and Morgan Sweet have been used as stembuilders have been examined, and these do not show canker lesions to any greater extent than other varieties. It would seem that, while they are susceptible to apple canker in this part of the Long Ashton nurseries, once the trees have become established in the orchard no further trouble occurs.

TABLE I.

SIX STEMBUILDERS ON TWO ROOTSTOCKS, TWO YEARS AFTER BUDDING.

Rootstock	Stem varieties	Total number of trees	Number of trees over 6ft. 6in.	Number of trees with apple canker lesions
Crab C	Bulmer's Norman ..	35	23	17
	Crab C	39	39	1
	Morgan Sweet ..	34	4	8
	Sweet Coppin ..	37	13	2
	Tremlett's Bitter ..	35	21	6
	Yarlington Mill ..	32	21	1
Seedling Cider (Knotted Kernel)	Bulmer's Norman ..	16	11	3
	Crab C	20	19	1
	Morgan Sweet ..	16	9	2
	Sweet Coppin ..	19	15	0
	Tremlett's Bitter ..	17	12	4
	Yarlington Mill ..	18	9	1

Two of the varieties resistant to canker, namely Yarlington Mill and Sweet Coppin, failed to produce a sufficient proportion of trees with stems thick enough to be high-worked two years after being budded. Both varieties are, however, capable of producing a 6 ft. 6 in. stem strong enough to support themselves.

Tremlett's Bitter proved completely unsatisfactory. In addition to its susceptibility to apple canker, the growing point died in almost 50% of the trees and, in consequence, there is now a very obvious bend in the middle of the stem of affected trees.

Discussion.

From this trial it would appear that only Crab C promises any improvement on the varieties at present in use as stembuilders. It will be seen from Table I that not only has it produced the greatest proportion of trees tall enough to be worked at the end of two years, but also that the number of trees surviving the attacks of canker is greater than for any other variety ; it is also resistant to apple mildew.

Arrangements have been made for a number of trees with Crab C, Bulmer's Norman and Morgan Sweet stems to be planted out near Taunton to study their orchard performance. The stems have been grafted at 6 ft. 6 in. with Dabinett and Yarlington Mill, two varieties of widely differing habits of growth. Records of cropping and vigour will be taken and a note made of rogue shoots arising from the stems. One of the criticisms of Crab C that may be put forward is that such shoots will produce crab apples, whereas similar shoots of Bulmer's Norman or Morgan Sweet will produce saleable cider fruit. Trees with a trunk of Crab C that were planted at Long Ashton twenty years ago have, however, shown no tendency to throw shoots from below the union, so this criticism may be unfounded.

Acknowledgments.

I am indebted to Mr. G. H. Jones for the photographs and to Mr. E. W. Hobbis for his advice and interest in this work.

REFERENCES.

(1) Hatton, R. (1929). Masters Memorial Lecture. Stock : scion relationship. *J. R. hort. Soc.*, **55,** 169–211.

(2) Swarbrick, T. (1928). Some preliminary observations upon the influence of the scion variety upon the root growth of young apple trees. *Ann. Rep. Long Ashton Res. Sta.*, 1927, 42–49.

(3) Barker, B. T. P. (1937). *Cider Apple Production.* Bull. No. 104. Ministry of Agriculture and Fisheries, 25.

VIRUS DISEASES OF FRUIT TREES: V. EXPERIMENTS ON RUBBERY WOOD, MOSAIC AND FLAT LIMB OF APPLES.

By L. C. LUCKWILL.

The last report on apple virus work at Long Ashton was published in 1954 (1). The present paper summarizes the work done on this subject during 1954 and 1955.

Virus content of clonal rootstocks in relation to stock size.

All the clonal apple rootstocks propagated at Long Ashton, with the exception of M.II, are now known to be infected to a greater or lesser degree with the virus that, in Lord Lambourne and some other varieties, produces symptoms of rubbery wood. The stocks are usually regarded as symptomless carriers in that, although infected, they do not show typical symptoms of rubberiness. It is possible, nevertheless, that such latent infection may have an adverse effect on the general vigour of the stocks. If this were so it would be expected that within any particular clone the smaller stocks would, on the

TABLE I.

INCIDENCE OF RUBBERY WOOD VIRUS IN LARGE AND SMALL GRADES OF CLONAL APPLE ROOTSTOCKS, AS DETERMINED BY INDEXING WITH LORD LAMBOURNE.

Stock	Large or Small	Numbers of trees						Average score	% rubbery
		Total	0*	1	2	3	4		
M.I ..	L	15	1	0	3	10	1	3·7	93·5
	S	18	1	3	9	5	0	2·0	94·5
M.IV ..	L	16	9	4	3	0	0	0·6	18·7
	S	8	2	3	3	0	0	1·1	37·5
M.VII ..	L	18	11	3	4	0	0	0·6	22·2
	S	16	6	1	6	3	0	1·0	56·1
M.IX ..	L	14	0	0	14	0	0	2·0	100·0
	S	15	0	0	3	4	8	3·3	100·0
M.XII ..	L	15	14	1	0	0	0	0·1	0·0
	S	18	8	4	2	0	0	0·4	11·1
M.XVI ..	L	20	20	0	0	0	0	0·0	0·0
	S	13	6	3	4	0	0	0·8	30·8
F.11 ..	L	17	7	1	9	0	0	1·1	52·8
	S	13	1	2	8	2	0	1·8	77·0
G.7 ..	L	17	5	0	12	0	0	1·4	70·5
	S	19	3	1	15	0	0	1·6	79·0

* 0=healthy; 1=doubtful; 2=definitely rubbery; 3=severely rubbery; 4=extremely rubbery (trees prostrate).

average, have a higher virus content than the larger ones. In order to investigate this point the following experiment was carried out.

Stocks of M.I, IV, VII, IX, XII and XVI and of the Long Ashton clonal selections F.11 and G.7, taken from the Long Ashton stoolbeds, were graded into size categories and 20 of the largest and smallest individuals in each clone were selected. These were lined out in runs of 10, and were budded in July 1953 with Lord Lambourne material taken from trees known to be free of rubbery wood ; bud failures were grafted with scions from the same trees in April 1954. At the end of 1955, when the trees were two years old, they were scored for rubberiness on an arbitrary scale ranging from 0 for " healthy " to 4 for " extremely rubbery," and an average score, giving a measure of the extent and severity of infection, was calculated for each group of trees. The results are presented in Table I, together with an estimate of the percentage of trees which could be classed as definitely rubbery. In calculating this percentage, trees in the " doubtful " category (score 1) have been classed as healthy.

It appears from Table I that with the exception of M.I and M.IX, both of which showed complete or almost complete infection, the incidence of rubbery wood virus was consistently higher in the low grade stocks than in the high grade stocks selected from each clone. This difference is reflected both in the average score for rubberiness and in the percentage of two-year old trees classed as definitely rubbery.

One possible explanation of this association between stock size and virus infection has already been advanced, viz. that the virus, although usually considered as latent (i.e. symptomless) is actually reducing the vigour of the stocks. Alternatively it could be postulated that the actual virus content of the large and small stocks is the same but that, owing to their smaller volume, the concentration of virus particles is highest in the smallest stocks. There would seem to be some support for this second hypothesis in the observation that the rubbery symptoms in infected Lord Lambourne are invariably most

TABLE II.

MEAN TREE WEIGHTS IN GM. CLASSIFIED ACCORDING TO STOCK SIZE AND VIRUS INFECTION, VAR. LORD LAMBOURNE.

	Healthy		Rubbery	
	Large	Small	Large	Small
M.I	120	120	104	91
M.IV	171	200	127	133
M.VII ..	280	180	60	145
M.IX	—	—	94	68
M.XII ..	304	316	—	300
M.XVI ..	327	193	—	130
F.11	177	280	167	168
G.7.....	252	193	163	151

severe under conditions where vegetative growth is restricted, e.g. in trees worked on M.IX or grown in pots.

In order to obtain information on the effect of stock size and virus infection on tree vigour all the trees were cut off at the union and weighed individually at the end of their second year of growth. The data for mean tree weight, classified according to health and stock size, are given in Table II. In arriving at these means only the weights of budded trees have been used ; in addition, trees classed as doubtfully rubbery have been excluded. Owing to the widely different numbers of trees falling into each category (from 0 to 20) statistical analysis of these data was not attempted.

It can be seen from Table II that stock size alone had no consistent effect on the size of the two-year old trees. Thus, considering only the healthy trees, it will be noticed that in three of the eight stocks the trees on the small stocks were actually larger than those on the large stocks ; in three other stocks the reverse was true, and in one there was no difference. The remaining stock (M.IX) produced no healthy trees so that the effect of stock size could not be judged. These results are therefore in agreement with the observations of Cannon (2) who also found that the diameter of the rootstock and the amount of rooting had no apparent influence on the size of nursery trees. Infection with rubbery wood virus, on the other hand, clearly reduced the vigour of the trees on all the stocks investigated, irrespective of stock size. A combined figure for all the stocks indicates that the weight of the rubbery trees was, on the average, 39% less than that of the healthy ones.

Transmission of rubbery wood from other varieties.

Although the rubbery wood condition is most characteristic of the variety Lord Lambourne, similar symptom patterns are occasionally seen on other varieties. In 1945 two 20-year old trees of James Grieve, a parent of Lord Lambourne, growing in an orchard in Worcestershire, were observed to be showing severe symptoms of what appeared to be rubbery wood. Scions from these trees worked on seedling rootstocks and grown at Long Ashton have continued to show these symptoms over the past ten years. Somewhat similar symptoms have been seen for a number of years on a tree of Bulmer's Norman in the cider orchard at Long Ashton. In order to find whether these symptoms were of virus origin and were identical with the rubbery wood of Lord Lambourne the following transmission experiment was carried out.

120 trees of each of the varieties Lord Lambourne, James Grieve and Bulmer's Norman were raised by budding on seedling rootstocks in 1953. The bud sticks were taken from trees known to be free of symptoms of rubbery wood. Below each bud and on the same side of the stock was placed a second " infection bud " taken from either a rubbery or a healthy tree. During the following growing season these lower buds were kept pinched back, only the upper buds being allowed to develop. Owing to lack of maiden growth no bud wood could be obtained from the rubbery trees of Bulmer's Norman and James Grieve and infection from these trees was carried out by means of bark grafts inserted just below the growth bud. Records were taken in September 1955 when the trees were two years old.

The results of this experiment, presented in Table III, show that rubbery wood from James Grieve was transmitted to Lord Lambourne by bark grafting

in 18 out of 19 trees. The symptoms produced in the Lord Lambourne were particularly severe ; the trees were dwarfed and the weight of a single apple was sufficient to cause them to become prostrate. Similar grafts produced rubbery symptoms, though less severe than those described above, on 12 out of 18 trees of James Grieve. A similar percentage of rubbery James Grieve trees was obtained by infection with buds from rubbery Lord Lambourne. By contrast no definitely rubbery trees of Bulmer's Norman resulted from infection by bark graft from rubbery James Grieve, or by bud from rubbery Lord Lambourne. Neither were the symptoms of rubberiness from the original orchard tree of Bulmer's Norman transmitted to the nursery trees of the same variety, or to Lord Lambourne or James Grieve. It must be concluded, therefore, that the apparent rubbery condition of Bulmer's Norman was due to some cause other than virus infection. The rubbery condition in James Grieve, on the other hand, seems to have been due to the same virus that causes rubbery wood in Lord Lambourne.

TABLE III.

ATTEMPTED TRANSMISSION OF RUBBERY WOOD BETWEEN LORD LAMBOURNE, JAMES GRIEVE AND BULMER'S NORMAN.

Scion variety	Source of inoculum	Inoculated by	No. of trees	No. of trees		
				Healthy	Doubtful	Rubbery
Lord Lambourne	Healthy Lambourne	Bud	19	19	0	0
	Rubbery „	Bud	17	1	7	9
	Healthy Grieve	Bud	17	15	2	0
	Rubbery „	Bark graft	19	1	5	13*
	Healthy Bulmer's	Bud	18	18	0	0
	Rubbery „	Bark graft	18	18	0	0
James Grieve	Healthy Lambourne	Bud	17	15	2	0
	Rubbery „	Bud	20	4	6	10
	Healthy Grieve	Bud	17	15	2	0
	Rubbery „	Bark graft	18	6	1	11
	Healthy Bulmer's	Bud	19	18	1	0
	Rubbery „	Bark graft	20	18	2	0
Bulmer's Norman	Healthy Lambourne	Bud	16	15	1	0
	Rubbery „	Bud	15	12	3	0
	Healthy Grieve	Bud	19	19	0	0
	Rubbery „	Bark graft	18	18	0	0
	Healthy Bulmer's	Bud	20	20	0	0
	Rubbery „	Bark graft	18	18	0	0

* The rubbery symptoms on these trees were particularly severe.

Symptomless carriers of apple mosaic.

In the previous paper in this series (1) it was reported that certain apple varieties which had been artificially infected with the vein-banding strain of apple mosaic had failed to develop symptoms, thus suggesting that they might be acting as symptomless carriers of the disease. This has now been confirmed

by a subsequent experiment in which buds from these symptomless trees were worked back on to healthy indicator plants of Lord Lambourne growing on seedling stocks. The bud infection was carried out in the summer of 1954, and during the following growing season all the indicator plants developed symptoms of mosaic. The varieties shown to be capable of acting as symptomless carriers of this disease are :—

Ellison's Orange.
Rival.
Tydeman's Late Cox.
Grenadier.
Sweet Coppin.
Ashton Brown Jersey.

The trees of St. Cecilia, mentioned in the previous list, died before infection buds could be taken from them, but it seems probable that this variety also may be classed as a symptomless carrier.

Transmission experiments with flat limb.

In 1944 Wallace, Ogilvie and Swarbrick (2) described a condition, prevalent on Lord Lambourne trees in this country, characterised by the flattening of the main limbs of the tree. They called this condition " flat limb " on the assumption that it was similar to the " flat limb " that occurs widely on the variety Gravenstein in North America and Australia. The latter condition has been shown to be transmissible from diseased to healthy trees by means of grafting and is therefore presumably of a virus nature (4). Successful attempts to transmit it to other varieties have not so far been reported, though there is evidence that certain varieties and stocks may be symptomless carriers, since healthy Gravenstein worked on them develops symptoms of the disease.

The symptoms of flat limb on Lord Lambourne differ somewhat from those on Gravenstein. In Lambourne the flattening is most marked at the base of the main scaffold limbs and is almost always in a vertical plane. In Gravenstein the flattened portions may occur anywhere along a branch, although they are most marked on the older portions, and do not appear to be orientated in any particular direction. There thus seemed some reason to doubt whether the flat limb condition of Lord Lambourne was, in fact, identical with the condition already described on Gravenstein. In order to obtain further information on this point the following experiment was performed.

In 1949 buds were taken from healthy and flat-limb trees of the variety Labygaard, a Gravenstein type growing in the National Fruit Trials at Wisley, and worked on to seedling rootstocks growing at Long Ashton. In the spring of 1951 the maiden shoots were cut back to 6 in. above the union and top worked with either healthy Labygaard or healthy Lord Lambourne scions. There were originally 14 trees in each category, but at the end of 1953 the plot was cleared and many of the trees destroyed, no symptoms having developed on the scions up to that date. However, 12 trees were saved and planted out on another plot for further observation and by the end of 1955 four of these trees were showing pronounced symptoms of the Gravenstein type of flat-limb. The records are summarised in Table IV from which it will be seen that only two of the four Labygaard trees worked on intermediate pieces taken from diseased trees developed symptoms. This suggests that not all the buds

TABLE IV.

TRANSMISSION OF GRAVENSTEIN " FLAT LIMB."
ALL TREES WERE WORKED ON SEEDLING ROOTSTOCKS AND HAD INTERMEDIATE STEM PIECES OF EITHER HEALTHY OR DISEASED LABYGAARD.

Intermediate stem of var. Labygaard	Top scion originally healthy	Total No. trees	No. trees showing flat-limb 1955
Healthy	Labygaard	4	0
Flat-limb	Labygaard	4	2
Healthy	Lambourne	2	0
Flat-limb	Lambourne	2	2

collected from the diseased tree were actually carrying the flat-limb virus, and may account for the difficulty in transmitting this disease by budding that has been reported by other workers (4). The appearance of symptoms on both of the Lord Lambourne trees worked on diseased intermediate stem pieces is of particular interest since, as far as can be ascertained, this is the first record of the Gravenstein type of flat-limb having been transmitted to another variety. The symptoms that developed on Lord Lambourne were similar to, though somewhat more severe than those shown by the comparable trees of Labygaard. On four-year old wood there was marked local flattening of the branches whilst on the main stem (five-year old wood) the flattening had developed into deep longitudinal depressions Fig. 1 (*a* & *b*). Only very slight symptoms could be found on three-year old wood and none at all on two-year or maiden growth. This symptom pattern is entirely distinct from the commonly occurring flat limb of Lord Lambourne Fig. 1 (*f*), which must therefore be regarded as a different condition and which, to avoid confusion, should be given a different name. The term " *oval limb* " might perhaps be applied to describe the common condition of Lord Lambourne, the virus nature of which has not yet been established.

Summary.

(1) The incidence of rubbery wood virus in large and small apple rootstocks selected from eight different clones has been investigated. Where infection of the clone was only partial the virus content of the small stocks was higher than that of the large stocks selected from the same clone.

(2) Stock size itself appeared to have no consistent effect on the weight of the two-year old tree, but infection with rubbery wood virus reduced the average tree weight by 39%.

(3) Rubbery wood has been transmitted by grafting from James Grieve to Lord Lambourne and in the reverse direction. A condition resembling rubbery wood in Bulmer's Norman was found not to be graft transmissible.

(4) Six apple varieties are listed which can act as symptomless carriers of the vein banding strain of apple mosaic virus.

(5) Gravenstein " flat-limb " has been transmitted by top-grafting from the variety Labygaard to Lord Lambourne. The symptoms are distinct from the flat-limb commonly occurring on Lord Lambourne. It is suggested that the latter condition should be distinguished by the name " *oval limb.*"

PLATE II

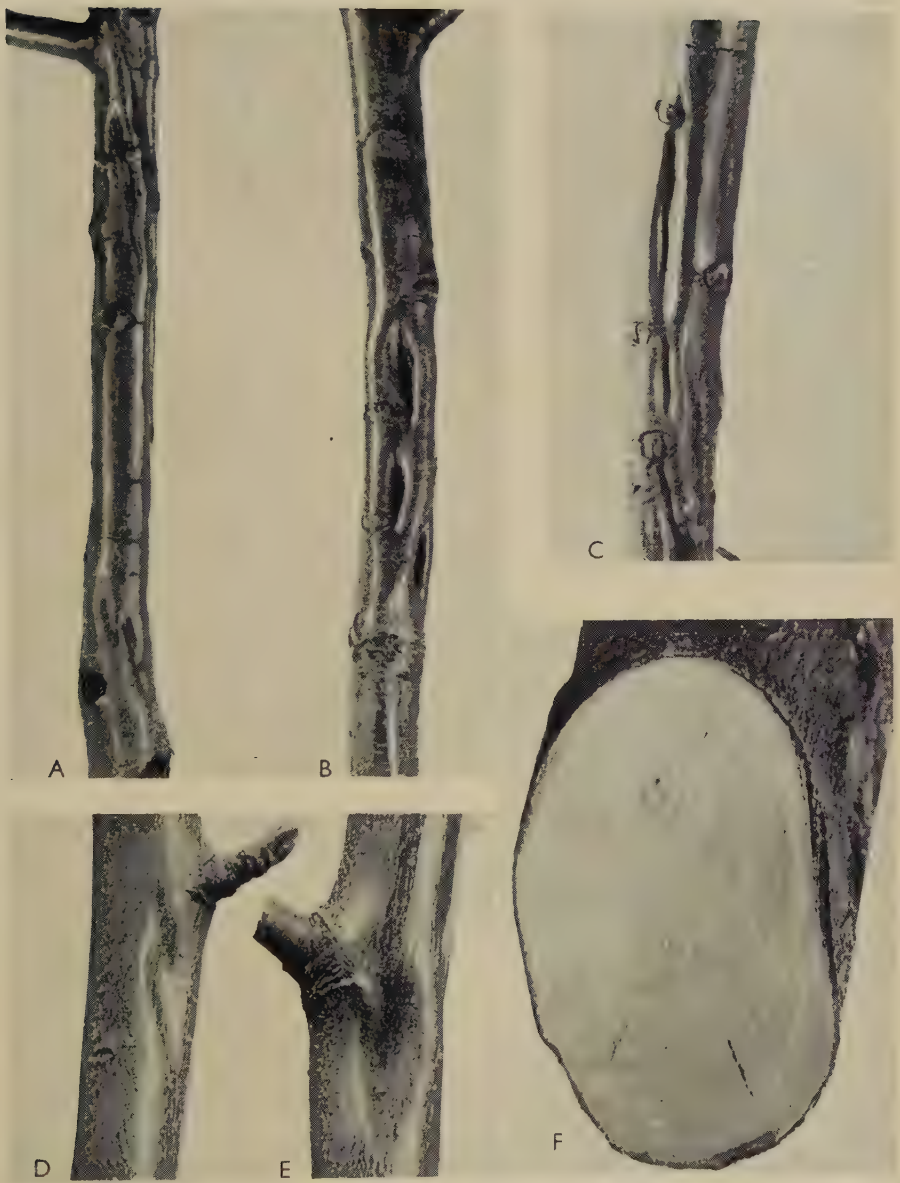


Fig. 1. (*a*) Gravenstein type of flat-limb on main stem of five-year-old tree of Labygaard : (*b*) Flat-limb symptoms developed on five-year-old stem of Lord Lambourne following grafting on to infected Labygaard : (*c*) Symptoms of Gravenstein flat-limb on four-year-old wood of Labygaard : (*d*) and (*e*) Symptoms of Gravenstein flat-limb on three-year-old wood of Lord Lambourne : (*f*) Section of branch of Lord Lambourne showing the condition commonly referred to as flat-limb.

Acknowledgments.

My thanks are due to Miss B. A. Rake and to Mr. A. I. Campbell, who looked after these experiments during my absence from the country.

REFERENCES.

(1) Luckwill, L. C. (1954). Virus diseases of fruit trees : IV. Further observations on rubbery wood, chat-fruit and mosaic in apples. *Ann. Rep. Long Ashton Res. Sta.*, 1953, 40–46.

(2) Cannon, M. B. (1941). Studies on the variation of nursery fruit trees on vegetatively raised rootstocks. *J. Pomol.*, **19**, 2–33.

(3) Wallace, T., Swarbrick, T. and Ogilvie, L. (1944). Some new troubles in apples with special reference to the variety Lord Lambourne. *The Fruit Grower*, **98**, 427. *The Grower*, **22** (49), 12–13.

(4) Hockey, J. F. (1943). Mosaic, false-sting and flat limb of apple. *Sci. Agric.*, **23**, 633–46.

GROWTH SUBSTANCES AS FRUIT-THINNING AGENTS FOR APPLES: PROGRESS REPORT.

By D. L. ABBOTT.

Introduction.

Experience in the use of NAA as a post-blossom thinning agent for apples at Long Ashton has indicated this compound to be unsuitable for general use. Results have been variable both between varieties and between years for the same variety. The desired degree of thinning has often been obtained, but although this has usually been accompanied by an increase in mean fruit diameter at harvest, the total marketable crop has been reduced. Nor has treatment with NAA resulted in increased blossom or fruit set in the following year but, since limbs and not whole trees have been used as experimental units in all the trials carried out, the possibility of an interaction between treated and unsprayed limbs cannot be entirely ruled out.

The reduced yield following treatment, without any apparent commensurate increase of growth or cropping the following year, has been attributed to a damaging effect of NAA upon the tree. This, at least in part, may be due to reduced photosynthetic activity of the sprayed leaves which, typically, soon show epinastic drooping and distortions, and a cessation of further growth of new leaves at the higher spray concentrations.

Hoffman, Edgerton and Fisher (1) have recently reported successful thinning by the use of naphthaleneacetamide. This compound is less drastic in its action than the acid, and has been used at concentrations as high as 80 p.p.m. by the authors with no apparent foliage damage when the spray was applied at early stages of development. In view of this report it was decided to carry out a small trial to assess the possible value of naphthaleneacetamide as a post-blossom thinning agent for apples under the conditions prevailing in this country.

Method.

Naphthaleneacetamide, formulated as a dry wettable powder, was applied to individual limbs of the varieties Crawley Beauty and Laxton's Superb as an aqueous suspension at concentrations of 25, 50 and 75 p.p.m. Control branches were not sprayed. Two opposite branches of 12 mature trees of each variety were used, affording 6 replicates of each treatment. In order to allow for possible interaction within a tree, the treatments were arranged so that each of the 6 possible combinations of the 4 treatments occurred once in each of two blocks.

The branches of Crawley Beauty were sprayed four days after petal-fall and those of Laxton's Superb seven days after petal-fall. On both occasions the weather was fine and warm.

Results.

Following application of the sprays, the leaves appeared quite normal and no drooping could be seen. After two weeks, however, the sprayed branches of Crawley Beauty could be easily identified by the large number of small fruitlets still attached to the branches. These had ceased to swell when about 5 mm. in diameter and, though those on the branches sprayed at 25 p.p.m. soon abscissed, many on those branches sprayed at 50 and 75 p.p.m. remained firmly attached nearly till harvest. A difference in leaf coverage was also observed two weeks after treatment. New leaves were unfolding and expanding on control branches and those sprayed at 25 p.p.m., but 75 p.p.m. appeared to have inhibited any development of new leaves so that these branches were very thinly covered and remained so throughout the season. Branches sprayed at 50 p.p.m. were intermediate in this respect.

Laxton's Superb showed no effect of treatment on either fruit or leaf development.

At harvest, fruit from each branch was weighed and graded by size. All the fruit from each treatment was then bulked for general observation of colour, maturity etc.

TABLE I.

SUMMARY OF HARVEST DATA.

Variety	Treatment	Mean wt. fruit/100 blossom clusters (kg.)	Mean no. fruit/100 blossom clusters		
			Total	Unmarketable ($<2\frac{1}{4}''$)	Marketable ($>2\frac{1}{4}''$)
Crawley Beauty ..	Control	5·14	98	91	7
	25 p.p.m.	3·82	49*	25	24**
	50 „	1·35	16**	6	10
	75 „	0·58	7**	2	5
Laxton's Superb ..	Control	2·98	55	50	5
	25 p.p.m.	3·53	57	42	15
	50 „	3·23	53	40	13
	75 „	2·93	49	38	11

Only columns 4 and 6 analysed statistically.
* Differs from control at 1% level of significance.
** „ „ „ „ 0·1% „ „ „

Table I shows that naphthaleneacetamide at 25 p.p.m. effectively thinned Crawley Beauty and considerably increased the marketable crop. At 50 and 75 p.p.m. the fruitlets were over-thinned and this resulted in an increase only in the proportion of larger fruits. All treatments were without effect on number or size of fruits on Laxton's Superb.

These preliminary results suggest that the amide is not fundamentally different from the acid in its effects, thinning being accompanied by a reduction in leaf cover and a considerable decrease in total yield. Nevertheless the success of naphthaleneacetamide at 25 p.p.m. in increasing the marketable crop from Crawley Beauty merits further trials with this compound.

Summary.

(1) Naphthaleneacetamide was applied as an aqueous spray at concentrations of 25, 50 and 75 p.p.m. to branches of Crawley Beauty 4 days after petal-fall and to Laxton's Superb 7 days after petal-fall.

(2) All treatments thinned Crawley Beauty with a consequent increase in the proportion of large fruit at harvest. The spray at 25 p.p.m. effected an increase in the marketable crop.

(3) The treatments were without effect on the number or size of fruits of Laxton's Superb.

Acknowledgments.

Thanks are due to the American Chemical Paint Company for the generous gift of the naphthaleneacetamide. Thanks are also due to Mr. G. M. Clarke for advice in the design of the trial and for the statistical analysis of the results, and to Miss A. J. Beagles for assistance in spraying.

REFERENCE.

(1) Hoffman, M. B., Edgerton, L. J. and Fisher, E. G. (1955). Comparisons of naphthaleneacetic acid and naphthaleneacetamide for thinning apples. *Proc. Amer. Soc. hort. Sci.*, **65,** 63–70.

A NOTE ON TEN YEARS CROPPING OF BLACK CURRANTS AT LONG ASHTON.

By E. W. HOBBIS and E. CATLOW.

During the summer of 1955 the tenth successive crop was picked from seven acres of black currants growing at Long Ashton. The early cropping of these bushes was reported upon in the Annual Report for 1947 (1).

Six varieties were grown, and during the ten years all but Malvern Cross were used for a number of experiments : Mendip Cross and Cotswold Cross for manurial experiments in which various treatments with organic manures were employed (2), (3), (4) ; Baldwin for experiments on levels of nitrogenous manuring ; Baldwin and Seabrook's Black for experiments on the incidence and control of Leaf Spot (*Pseudopeziza ribis*) (5) ; Baldwin, Seabrook's Black and Westwick Choice for experiments to delay flowering by the use of chemical sprays (6).

All varieties except Malvern Cross, therefore, have been subjected to various experimental treatments, some of which might have been expected to promote heavy cropping and others to reduce potential cropping to some degree.

General Management. All bushes were grown under systems of clean cultivation, with or without annual mulching along the rows in accordance with the experimental treatments. For two seasons the area under Baldwin was divided into 12 blocks each of 2 plots, half of which were mulched with straw at 6 tons per acre and the remainder cultivated without mulching. Manuring varied according to experimental requirements but generally the annual application of nitrogen was from 4 -6 cwt. of a nitrogenous fertilizer, with potash and phosphate at 1 and 2 cwt. respectively ; all were applied in March except that a 6 cwt. dressing of nitrogenous fertilizer was given in two equal applications, the first in March and the second in May.

The general aim in cultivation was to keep the plots free of weeds with the minimum of root disturbance. Hand hoeing was done with push hoes of the Dutch type throughout and inter-row cultivations were as shallow as possible, using in the early years spring-tined cultivators and harrows and latterly a rotary-cultivator. No ploughing was done between the bushes throughout the period.

Pest and disease control measures conformed with good field practice. Winter spraying was followed by lime-sulphur in the spring of every second year during the first six years and this controlled Big Bud mite except in the variety Cotswold Cross, which developed a heavy infestation by the eighth year, followed by reversion. Annual spraying with lime-sulphur was given during the last four years.

Inspections for reversion were made twice each season, in spring and in June, when infected or suspect bushes were grubbed.

Crop Yields. Crop weights from all bushes were recorded each season and these have been arranged in Table I to show the yield of each variety irrespective of the different treatments given. The resulting figures are considered to reflect the level of cropping that might be expected under average conditions of field culture at Long Ashton.

TABLE I.

BLACK CURRANT YIELDS (TONS/ACRE) :
LONG ASHTON RESEARCH STATION 1946–55.

	Spacing 10′×3⅓′ (Bushes/acre 1,306)		Spacing 8′×4′ (Bushes/acre 1,361)			
Variety	Baldwin	Seabrook	Cotswold Cross	Malvern Cross	Mendip Cross	Westwick Choice
1946	1·13	1·06	2·23	1·82	1·98	0·88
1947	2·82	2·47	3·01	3·40	3·14	1·07
1948	0·62	2·36	2·99	3·01	3·29	2·24
1949	3·44	1·68	5·25	4·06	4·08	4·28
1950	4·34	3·87	3·26	5·13	3·34	2·79
1951	1·97	1·70	1·70	2·83	2·24	2·25
1952	2·92	1·98	1·57	2·95	4·13	2·78
1953	5·28	3·29	2·32	1·71	3·14	1·68
1954	1·85	2·58	1·43	1·39	2·24	2·93
1955	3·80	2·20	—	—	3·06	1·90
Annual Mean	2·82	2·32	2·64	2·92	3·06	2·28

In studying these figures, it is of interest to note that the average crop of black currants per acre in England and Wales during the period 1944–52 was 22·8 cwt., a yield considerably below the figure for Long Ashton.

Effect of Spacing on Yield. All black currants at Long Ashton were grown on the hedgerow system, at distances of 8 ft. × 4 ft. or 10 ft. × 3⅓ ft., requiring 1,361 or 1,306 bushes per acre respectively.

One of the advantages of the hedgerow system is that, given adequate distance between the row to accommodate appropriate machinery, it is possible, by close planting in the row, to plant a high number of bushes per acre. By so doing the total amount of wood produced is also high and so contributes to heavy crops, especially during the early years when the best possible return on capital outlay is necessary.

Cropping Life. The profitable life of a plantation has generally been accepted as 10 cropping years but provided the bushes are kept vigorous and free from pests and diseases, the yields shown in Table I can, with good management, be expected for a further five years. In exceptional cases plantations are known to have cropped profitably for upwards of 20 years.

Acknowledgments.

Thanks are due to Mr. R. M. Jarrett who assisted in crop recording throughout the period under review and to Mr. G. E. Clothier who carried out annual roguing for reversion.

REFERENCES.

(1) Hobbis, E. W. (1948). *Ann. Rep. Long Ashton Res. Sta.*, 1947, 43–52.
(2) Bould, C. and Catlow, E. (1947). *Ann. Rep. Long Ashton Res. Sta.*, 1946, 31–36
(3) —— and —— (1948). *Ann. Rep. Long Ashton Res. Sta.*, 1947, 52–58.
(4) —— and —— (1951). *Ann. Rep. Long Ashton Res. Sta.*, 1950, 49–53.
(5) Corke, A. T. K. (1954). *Ann. Rep. Long Ashton Res. Sta.*, 1953, 154–158.
(6) Ferres, Helen (1952). *Ann. Rep. Long Ashton Res. Sta.*, 1951, 40–42.

FRUIT DROP IN BLACK CURRANTS: I. FACTORS AFFECTING "RUNNING OFF."

By S. S. TEAOTIA and L. C. LUCKWILL.

Introduction.

Under conditions of good cultivation and in the absence of frost damage the initial set of fruit in black currants is high, in the sense that the ovaries of most of the flowers swell and develop into young berries. During the first five weeks following full bloom a proportion of these developing berries are shed, a phenomenon known to fruitgrowers as " running off." In addition to this early period of fruit drop the black currant also shows a well-marked " pre-harvest drop " of ripe berries.

In the early days of commercial black currant growing excessive " running off " often resulted in very low yields, frequently amounting to only a few hundredweights per acre. Since that time, the introduction of improved varieties, and a better understanding of the manurial and cultural requirements of this crop, have to some extent mitigated this trouble and have led to a general improvement in cropping. In spite of this improvement, fruit drop, particularly " running off," remains an important problem in black currant growing, and may still be a major factor limiting yield. " Pre-harvest drop " is not usually considered a serious source of loss in this crop, though it commonly accounts for 3 to 5% of berries, and a delay in picking of only one week may increase this to as much as 10 to 12%. Moreover, since the berries that drop at this time are the largest and most mature, the actual loss of crop would be greater than this.

No investigation of the physiological aspects of this problem seems to have been reported since the early work of Wellington, Hatton and Amos at East Malling, published in 1921 (5), though in recent years considerable progress has been made in the study of fruit drop in other crops. Wellington and his co-workers established that " running off " was not associated with reversion or with attack by Big-bud mite, as had been previously suggested, or primarily with weather, soil or manurial treatments. They attributed the phenomenon to inefficient pollination of the flowers towards the apex of the inflorescence, which, they thought, resulted from inadequate numbers of pollinating insects, combined with a successive lengthening of the style in successive flowers of the raceme, which effectively prevented self-pollination. In the present paper* further observations on fruit drop in black currants are reported, with particular reference to berry size, seed number, position in the truss and to pollination conditions.

Experimental.

The observations and experiments described below were made during the years 1951–4 on a seven-acre plantation of black currants at Long Ashton. The varieties Cotswold Cross and Mendip Cross were planted as two-year old bushes in 1944, and Westwick Choice in 1945, at a spacing of 8 × 4 ft., giving

* This paper incorporates part of a Ph.D. thesis presented to the University of Bristol in 1953 by S. S. Teaotia, a student of the Horticultural Science Laboratories.

a density of 1,361 bushes per acre. The plots of Baldwin and Seabrook's Black were originally laid out in 1941 at a spacing of 10 × 10 ft., but in 1944 were interplanted in the rows to give a spacing of 10 × 3⅓ ft. and a density of 1,306 bushes per acre. From 1944-50 the plots of Cotswold and Mendip Cross were used for an experiment to investigate the effect of various organic manures on growth and yield : details of treatments and results have been published (1). The bushes of other varieties have received annual dressings of farmyard manure, supplemented with NPK fertilizer supplying 4 to 6 cwt. nitrogen per acre. All varieties have received routine tar-oil winter washes and spring applications of lime-sulphur, and reversion has been kept in check by immediate grubbing of all infected bushes.

TABLE I.

FRUIT DROP OF BLACK CURRANT VARIETIES AT LONG ASHTON IN 1951.

Variety	Number of flowers per raceme		Number of fruits per raceme at harvest		Total fruit drop per raceme	
	Mean	S.E.	Mean	S.E.	No.	%
Seabrook's Black..	7·5	·19	3·6	·24	3·9	52·0
Westwick Choice	11·5	·66	5·8	·43	5·7	49·5
Baldwin	7·9	·61	5·0	·35	2·9	36·7
Mendip Cross	11·7	·32	7·5	·21	4·2	35·9
Cotswold Cross	10·5	·45	7·2	·61	3·3	31·4

Extent of fruit drop in different varieties.

The severity of fruit drop in black currants is to a large extent a varietal character, though it can be modified by other factors such as soil, climate and management. Varieties of the French group are usually regarded as particularly prone to " running off," and this is supported by records taken during 1951, when Seabrook's Black was found to drop over 50% of its berries. In Cotswold Cross " running off," although less serious, still accounted for the loss of over 30% of berries. The three other varieties investigated in 1951 showed intermediate amounts of drop (Table I). These figures are given to indicate the extent of the drop that can be expected in well-managed plantations though it should be noted that the comparisons between varieties are not strictly valid owing to the different past histories of the plots and to the fact that there are differences in the soils on which they were growing.

Seasonal distribution of drop.

In 1951 the seasonal distribution of drop in five varieties was investigated by counting all the berries on ten shoots of each, at intervals from fruit-set to harvest. The intensity of the drop in successive periods was calculated by expressing the number of fruits dropped as a percentage of the total number present at the beginning of the period.

At the time of the first count, on May 15th, very little drop had occurred in any of the varieties. All varieties showed a heavy loss of berries during the next period of 21 days, followed by a period of reduced drop until just before harvest, when the drop again increased. This pre-harvest drop of ripe berries was particularly severe in Baldwin, where it accounted for 12·9% of fruits (Table II).

TABLE II.

PERCENTAGE FRUIT DROP OF BLACK CURRANT VARIETIES DURING DIFFERENT PERIODS IN 1951.

Variety	May 15 — June 5	June 6 — June 19	June 20 — July 2	July 2 — harvest	Date of harvest
Seabrook's Black	42·2	6·7	1·8	6·1	July 10
Westwick Choice	39·9	9·7	4·6	6·0	July 23
Baldwin	25·4	1·7	3·0	12·9	July 18
Mendip Cross	21·2	13·5	2·8	3·5	July 12
Cotswold Cross	21·6	5·2	1·0	3·9	July 16

In 1952 more detailed records of fruit drop were made on Seabrook's Black by counting all the fruits on 15 shoots at weekly intervals from two weeks before bloom until harvest. The mean rate of fruit drop per day over each weekly period was calculated using the exponential formula proposed by Vyvyan (4). Similar counts were made on the same plot in 1954, and comparable records for 1953 are available from the work of Wright (6). The drop records for these three years (Fig. 1) show a close similarity, and indicate that, in this variety, " running off " consistently reaches its peak during the third week after full bloom. In 1953 and 1954 the drop actually started well before full bloom, the drops during this period consisting chiefly of immature flower buds. Some bud-shedding commonly occurs in black currants, but is not normally serious except in years when frosts occur immediately following the emergence of the inflorescence. The flower buds at this stage of development appear to be particularly susceptible to low-temperature injury, and in 1952 it was estimated that 9·0% of racemes in Westwick Choice were killed and a further 68% damaged, due to this cause. Some frost damage was also observed in this variety in 1951 and 1954.

The variety Seabrook's Black is normally picked at Long Ashton about nine weeks after full bloom. In 1953 harvesting of the experimental bushes was purposely delayed for two weeks, and the effect of this delay on losses from pre-harvest drop is strikingly illustrated in Fig. 1.

Effect of berry size and position in truss.

A random sample of 40 trusses of each of the five varieties studied was taken a week before harvest, and the berry drop from each position was recorded. All the berries remaining on the trusses were then weighed individually. In Fig. 2 the percentage drop and mean fruit weight are plotted against position

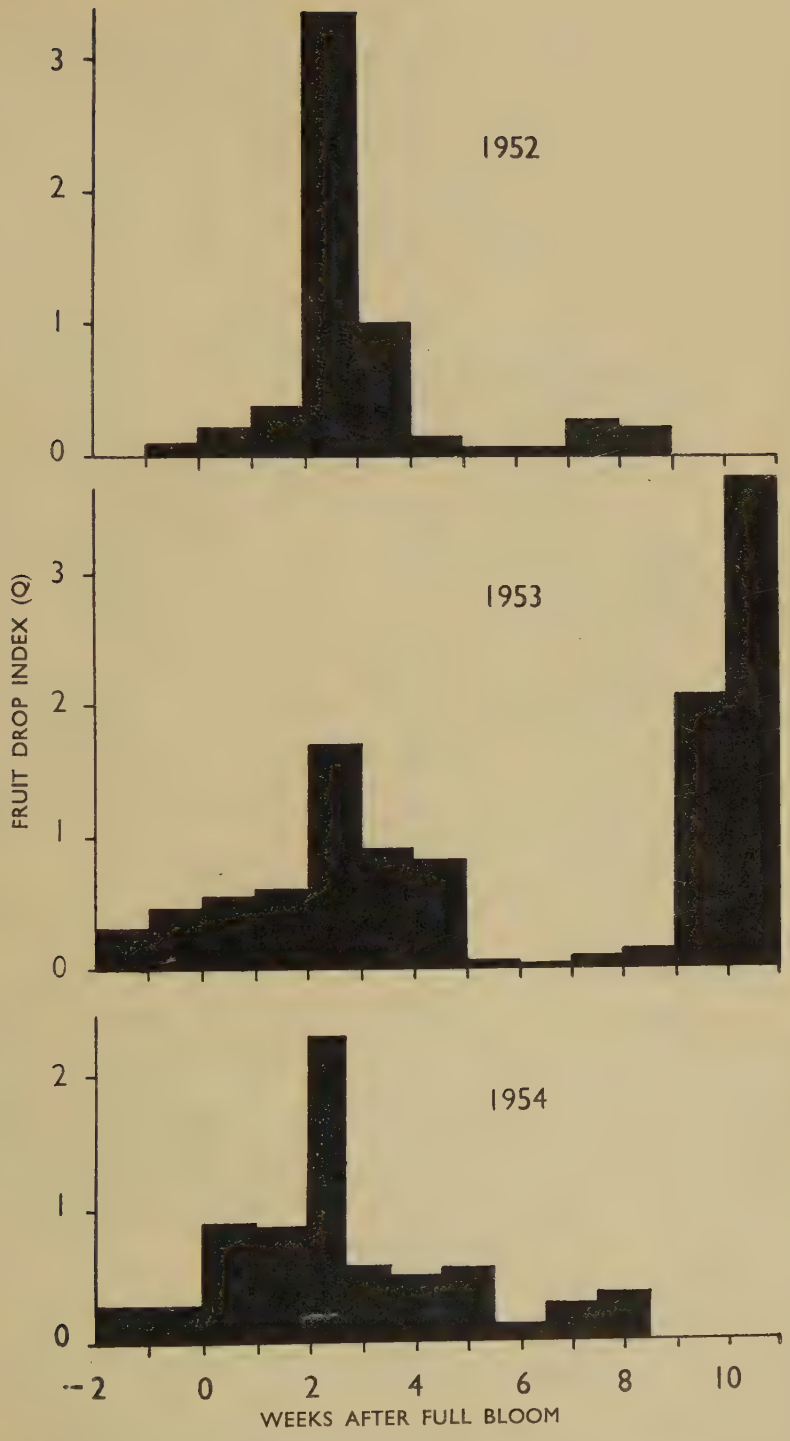


Fig. 1.

Fruit drop from black currant var. Seabrook's Black in three successive years.

Fig. 2

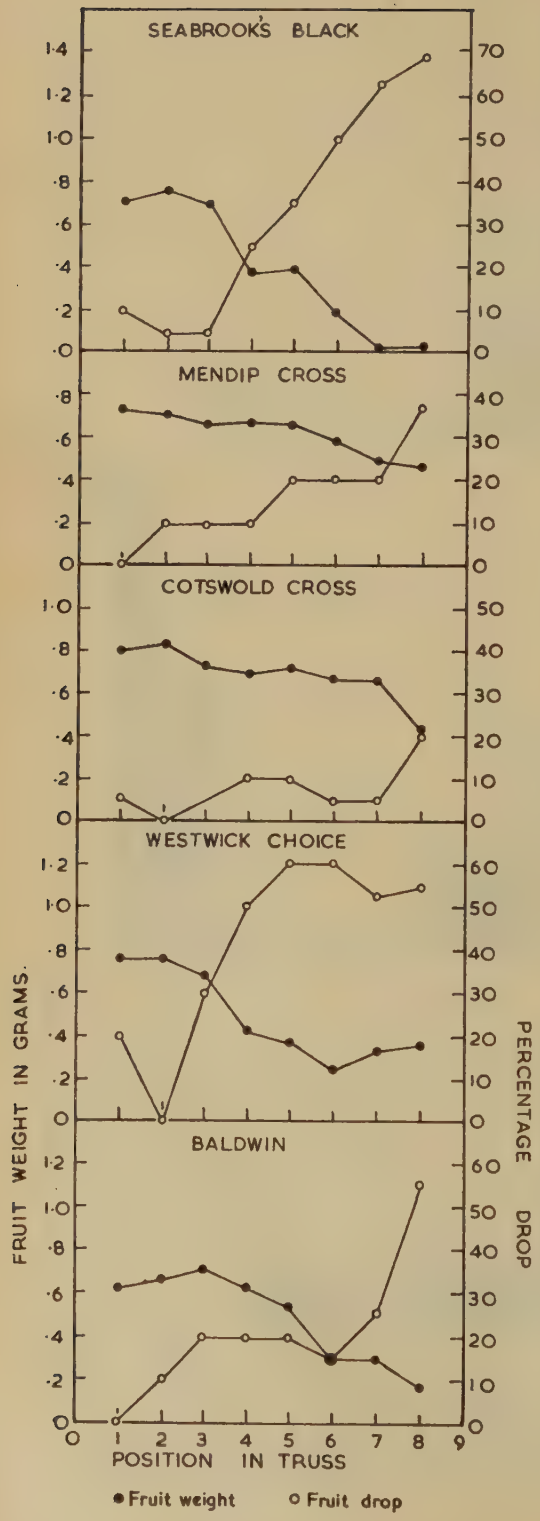


Fig. 3

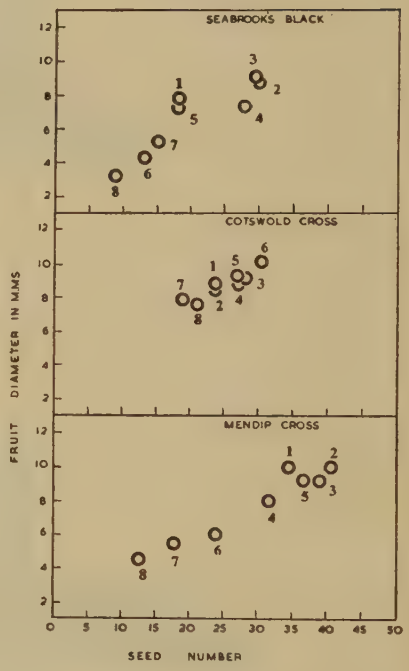


Fig. 2. Mean fruit weight and total fruit drop in relation to position of fruits in raceme. Number 1 position is basal.

Fig. 3. Correlation diagrams between seed number and fruit diameter at different positions in the raceme. Position in raceme is indicated by numbers, number 1 being the basal position.

in the truss, number 1 being taken as the basal position. In all varieties fruit drop increases and fruit weight decreases from the base towards the apex of the truss, though this tendency is much more marked in some varieties than in others. Cotswold Cross, which produces berries of relatively large and uniform size, shows less drop from the terminal portions of the truss than do varieties such as Seabrook's Black, where the range of berry size is greater. It appears therefore that the tendency to drop may be associated with small size of berry, rather than directly with position of the berry on the infructescence.

Fruit size and seed content.

Seeds are known to exert a controlling influence on the development of fruits, and in many plants high correlations between seed number and fruit size have been recorded. Hence, in an attempt to determine why in many varieties of black currant the berries towards the apex of the truss are smaller than those near the base, attention was first directed to their seed content.

Twenty unbranched fruit-trusses from each of the varieties Seabrook's Black, Mendip Cross and Cotswold Cross were picked a few days before the normal harvest date and berries up to the 8th position on the truss were removed and preserved in formalin-acetic-alcohol. After measuring the transverse diameter of each fruit, the seeds and pulp were extracted, placed in small muslin bags, and washed in running water to remove mucilage. The cleaned seeds were dried on blotting paper and counted, only fully developed seeds being included in the count. In Fig. 3 the mean fruit diameter (y) is plotted against mean seed number (x) for each position in the truss. It can be seen from the figure that there is a high correlation between these two variables, and that in Seabrook's Black and Mendip Cross both fruit diameter and seed number decrease towards the apex of the raceme. Cotswold Cross differs from the other varieties in that the range of fruit size and seed number is much smaller, and neither decreases markedly towards the apex.

TABLE III.

ANALYSIS OF COVARIANCE BETWEEN SEED NUMBER AND FRUIT DIAMETER, IN RELATION TO POSITION OF FRUIT IN RACEME.

Variety	Source of variation	d.f.	Coeff. of regression	Coeff. of correlation
Seabrook's Black	Between positions	7	0·232	0·94
	Within positions	152	0·241	0·98
Mendip Cross	Between positions	7	0·199	0·97
	Within positions	152	0·197	0·86

The correlation between fruit size and seed number at different positions in the raceme does not necessarily imply a causal relationship between these two variables. The correlation could result from the influence of some common factor associated with position acting independently on each variable. Thus, the terminal berries may be smaller and develop fewer seeds because they are less favourably located to receive elaborated food materials from the leaves

than are the basal berries. However, analysis of covariance of the data for Seabrook's Black (Table III) indicated that there is an equally high correlation between seed number and fruit diameter within any one position on the truss. The " within positions " correlation is also very high in Mendip Cross, showing that the relationship between seed number and berry size is largely a direct one, and is not due primarily to a position effect. It is of interest to note that in all three varieties the lowest berry on the truss is anomalous, in that it is larger than would be expected on the basis of its seed number (Fig. 3).

From the above observations and from what is known in other fruits, e.g. apple (2), regarding the importance of seeds as centres of auxin formation, it is concluded that, in the black currant, differences in seed number are the principal cause of variation in fruit size and in the amount of drop at different positions in the truss. In seeking further for the reason for this decrease in seed number towards the apex of the inflorescence, three possible explanations were considered, viz. :—

(i) The terminal flowers may have fewer ovules than the basal ones.
(ii) Pollination of the terminal flowers may be incomplete.
(iii) A higher proportion of seeds may abort before reaching maturity in the terminal as compared with the basal berries.

TABLE IV.

OVULE NUMBER AND SEED NUMBER IN RELATION TO POSITION IN RACEME. BLACK CURRANT VARIETY SEABROOK'S BLACK.

Position in raceme	Mean number of ovules per ovary	Mean number of seeds per fruit	Seed number as % of ovule number
1	124	18	14·5
2	146	31	21·2
3	149	30	20·1
4	149	29	19·7
5	132	19	14·4
6	130	13	10·0
7	126	15	11·9
8	122	9	7·4
9	118	—	—
10	121	—	—
11	106	—	—

Ovule number in relation to seed number.

The ovaries of all the flowers on five trusses of Seabrook's Black were dissected, and the number of ovules in each was counted under a low-power microscope. In Table IV the mean number of ovules in flowers at different positions is shown, together with a similar count of mature seeds made when the berries were ripe. These counts reveal that, even in the largest berries,

less than one quarter of the ovules develop into mature seeds : in the smaller berries towards the end of the raceme the proportion is of the order of one tenth. Thus, although there is some decrease in ovule number at successive positions in the raceme, this factor obviously cannot be considered as limiting seed number : even the flowers at the extreme tip of the truss, which rarely set fruit, have approximately four times the number of ovules required for developing an optimum content of 30 seeds.

Pollination experiments.

In an attempt to determine why so many ovules fail to develop into seeds attention was directed to the question of pollination, which Wellington and his co-workers considered the most important factor in " running off." If inadequate insect pollination is the reason for the low seed content and greater tendency to drop of the terminal berries, it should be possible to prevent this by hand pollination of the flowers. In 1952, therefore, an experiment was set up to compare the effect of hand and bee pollination on " running off " in Seabrook's Black. Vigorous three-year old bushes growing in pots were moved into the glasshouse about fourteen days before flowering. Two bushes were left uncovered to be pollinated by hive bees, a colony of which was moved into

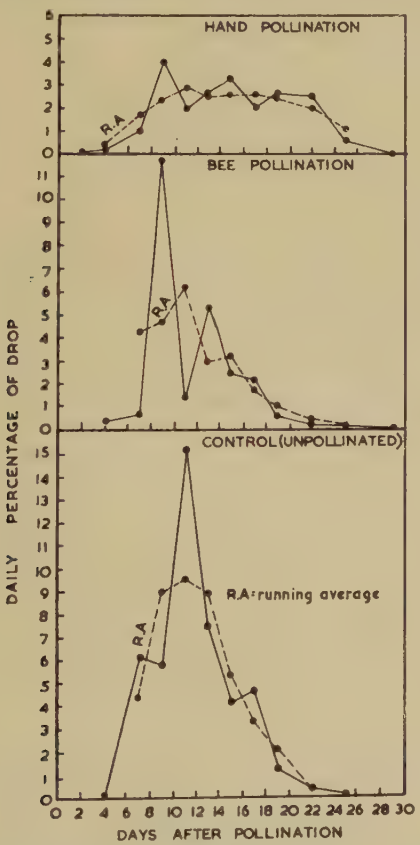

HAND POLLINATION
BEE POLLINATION
CONTROL (UNPOLLINATED)
R.A.
R.A: running average
DAILY PERCENTAGE OF DROP
DAYS AFTER POLLINATION
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30

Fig. 4. Fruit drop from bushes of Seabrook's Black kept in glasshouse, showing effect of different methods of pollination.

the house as soon as the flowers opened. Four other bushes were enclosed in cages, built of transparent sheet plastic and muslin, to protect them from bees. On two of these covered bushes all the flowers were hand pollinated with a camel-hair brush as soon as they opened, whilst the other two bushes were left to pollinate themselves. Dropped flowers and fruits were collected from each bush at two to four day intervals after flowering, and the mean percentage daily drop was calculated (Fig. 4). In all three treatments the drop reached its peak in the second week after flowering, as compared with the third week for bushes in the field. The bushes receiving neither hand nor bee pollination showed a very heavy drop, which reduced the final set to 7% of the number of flowers. The bee pollinated bushes also showed marked "running off," though the final set was increased to 48%. On the hand pollinated bushes the maximum rate of drop was much reduced, as compared with the bee pollinated ones, though the drop continued over a longer period. The final set of 53% did not differ significantly from that where bee pollination was employed.

TABLE V.

EFFECT OF SUPPLEMENTING NATURAL POLLINATION IN THE FIELD WITH HAND POLLINATION, ON THE SET OF TWO VARIETIES OF BLACK CURRANT.

Variety	Natural pollination		Natural + hand pollination	
	Number of flowers	% set	Number of flowers	% set
Seabrook's Black	1403	69·4	1569	73·6
Mendip Cross	1632	76·5	1679	78·4

In this experiment the concentration of bees around the bushes in the glasshouse was exceptionally high, and it was thought that under plantation conditions less efficient pollination conditions might prevail. An experiment was therefore carried out to determine the effect of supplementing natural field pollination with hand pollination. Three bushes each of Seabrook's Black and Mendip Cross were used for the experiment. All the open flowers on seven shoots on each bush were hand pollinated daily throughout the blossom period, and the final set of berries compared with that on a similar number of adjacent shoots which received only natural pollination. The results, shown in Table V, show no significant difference between the two treatments for either variety and it is clear that, under the conditions of this experiment, "running off" cannot be attributed to inadequate pollination. This was confirmed by microscopic examination of the berries dropped from the open pollinated bushes: in every instance an abundance of pollen tubes, far in excess of the number of seeds developed, was observed in the ovarian cavities of these abscissed fruits.

Seed abortion.

In 1951, during the peak period of berry drop, a collection was made from a bush of Seabrook's Black of berries which fell when the bush was shaken, and of berries which at that time were still firmly attached. Some of these were examined by dissection whilst others were fixed and embedded in paraffin wax for sectioning.

Examination of the fruits at this stage revealed that, in both attached and in dropped fruits, a far larger number of ovules are fertilized than ever develop into mature seeds; the remainder, after starting their development in an apparently normal manner, undergo abortion. In all fruits examined at this stage, a large number of these aborting seeds were found: many of them could not be distinguished superficially from normal seeds, though subsequent sectioning revealed that they contained collapsed endosperm tissue and embryos that had become disorganised at an early stage of development (Plate III). Other seeds were much smaller than normal and appeared shrivelled, indicating that abortion had occurred at an earlier stage. Owing to the difficulty of distinguishing with certainty between normal and abnormal seeds at this stage of development, no reliable counts could be obtained that would indicate the amount of seed abortion in different fruits on the truss. It is clear, however, that in black currants subjected to normal pollination conditions, the amount of embryo and endosperm abortion rather than the number of ovules fertilized is the principal factor regulating the final seed content of the berry.

Discussion.

The observations reported in this paper do not support the conclusions of Wellington, Hatton and Amos (5) that inefficient pollination is the primary cause of " running off " in black currants, since it is clear that the phenomenon still occurs even when every flower is individually hand pollinated. Although it is true that fruit drop is greatly increased under conditions of inadequate pollination, no evidence was obtained that such conditions prevailed in the Long Ashton plantations in 1952. It is possible that, in other locations or in other years, the natural population of pollinating insects might be insufficient to effect full pollination of all the flowers, and under such conditions the importation of hive bees during the flowering season would be expected to increase fruit set though not to eliminate " running off."

The berries that are shed during the " running off " period are actively growing fruits whose ovules appear, by microscopic observation, to have been fertilized in the normal manner. These berries differ from those that remain attached to the strig only in their smaller average size and lower seed content. In these respects " running off " of black currants is comparable with the so-called " June drop " of apples and other pome fruits.

The work of Wright (6) has shown that fruit abscission in the black currant is under the control of an auxin (*Ribes* auxin 2) which, he suggests, is formed as a by-product of the synthesis of reserve protein in the endosperm of the seed. Consequently, the lower the seed content of the fruit the smaller would be the flow of auxin to the abscission layer, and the greater the liability of the fruit to drop. The crux of the " running off " problem would therefore seem to lie in the factors responsible for the relatively low seed content of the berries towards the apex of the truss. The microscopic observations reported in this paper suggest that the most important factor involved here is abortion of a large proportion of the young seeds, rather than failure of fertilization. An essentially similar conclusion has been reached by Neumann (3) in a recent study of fruit development in the variety Laxton's Mite-free. Future work on this problem should therefore be directed to discovering the cause of seed abortion, which probably involves both hormonal and nutritional factors.

Summary.

(1) Black currants show two periods of fruit drop which together account for the loss of 30 to 50 per cent. of the berries. The most serious is the early drop (" running off ") which reaches its peak in the third week after bloom. Pre-harvest drop may cause serious losses if harvest is delayed.

(2) The berries towards the apex of the truss usually have fewer seeds and are more subject to " running off " than are those at the base. There is also a high correlation between seed number and berry weight, both between and within positions on the truss. Attention is drawn to the similarity between the " running off " of currants and the " June drop " of apples.

(3) " Running off " in Seabrook's Black is greatly increased in the absence of adequate pollination. However, even where pollination conditions are optimal (hand pollination of each flower), much " running off " still occurs. Lack of pollination cannot therefore be considered the primary cause of this phenomenon.

(4) On the average the dropped berries contain fewer good seeds than do those that remain attached. Their lower seed number results from abortion of a large proportion of the fertilized ovules at various stages of development.

REFERENCES.

(1) Bould, C. and Catlow, E. (1951). A manurial experiment on black currants. Progress Report III. *Ann. Rep. Long Ashton Res. Sta.*, 1950, 49–53.

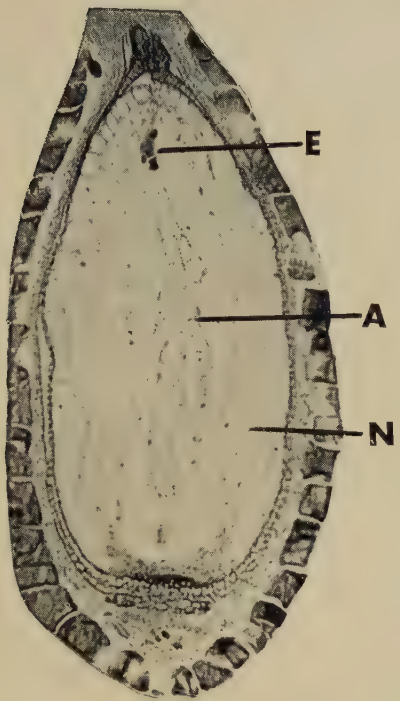
(2) Luckwill, L. C. (1953). Studies of fruit development in relation to plant hormones : I. Hormone production by the developing apple seed in relation to fruit drop. *J. hort. Sci.*, **28,** 14–24.

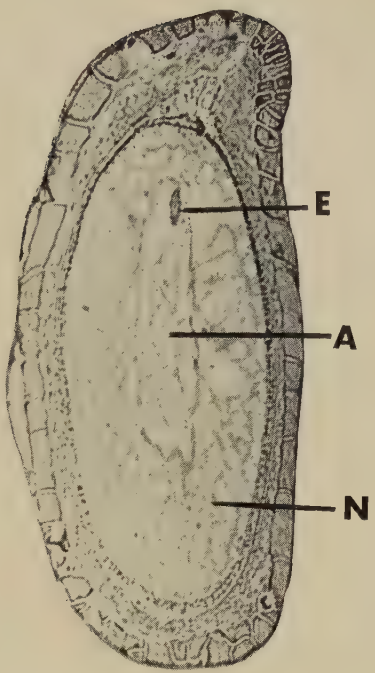
(3) Neumann, U. (1955). Über Beziehungen zwischen Wuchsstoffgehalt und Fruchtentwicklung bei Johannisbeeren. *Arch. Gartenbau*, **3,** 274–96.

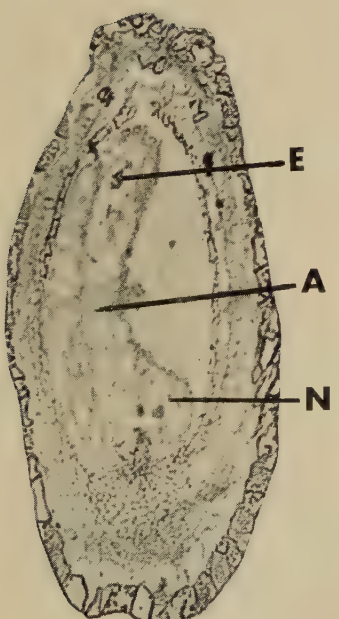
(4) Vyvyan, M. C. (1946). Fruit fall and its control by synthetic growth substances. *Imp. Bur. Hort. & Plant. Crops, Tech. Comm.* No. 18.

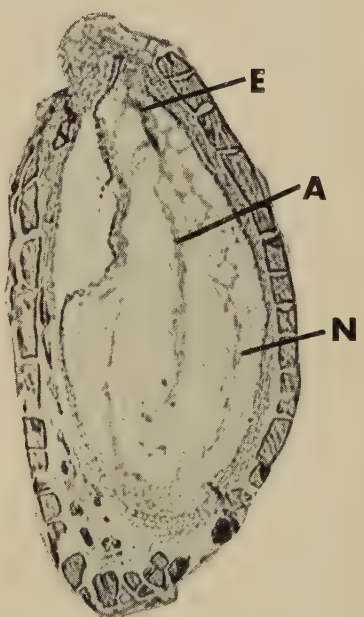
(5) Wellington, R., Hatton, R. G. and Amos, J. (1921). The " running off " of black currants. *J. Pomol.*, **2,** 160–198.

(6) Wright, S. T. C. (1954). A chromatographic study of auxins in relation to fruit morphogenesis and fruit drop in black currant (*Ribes nigrum*). Unpub. thesis, University of Bristol.









Longitudinal sections of developing seeds, approximately three weeks after fertilization, taken from fruits that had recently abscissed. Variety Seabrook's Black. *Above*: Two seeds showing apparently normal development. *Below*: Two seeds in which the endosperm tissue has collapsed and the embryo has become disorganised.
A=endosperm; E=embryo; N=nucellus.

FRUIT DROP IN BLACK CURRANTS: II. THE EFFECT OF NAPHTHALENE ACETIC ACID.

By L. C. LUCKWILL.

Introduction.

Since "running off" of black currants is basically an abscission phenomenon it was thought that it might be possible to prevent it, or at least reduce its intensity, by the use of synthetic growth regulators. A pilot experiment was carried out on Seabrook's Black in 1952 using sprays containing 1-naphthalene acetic acid (NAA) or 2:4 dichlorophenoxyacetic acid (2:4 D) applied at different stages of development (2). A significant reduction in drop was obtained with NAA at 10 parts per million applied just prior to the opening of the first flowers and, in view of this promising result, a large scale field trial was carried out at Long Ashton in 1954.

Experimental.

In this experiment a spray containing 10 parts per million NAA, and succinate wetter at the rate of 2 fl. oz. per 50 gallons, was applied with knapsack sprayers to bushes of Seabrook's Black and Westwick Choice on two different

TABLE I.

EFFECT OF NAPHTHALENE ACETIC ACID (10 P.P.M.) ON FRUIT DROP, BERRY SIZE AND CROPPING OF TWO VARIETIES OF BLACK CURRANT IN 1954.

	Control unsprayed	Early spray	Late spray
Seabrook's Black (Harvested July 12)			
Date of spraying	—	April 20	May 17
Early drop (as % no. of flowers)	31·2	29·4	43·6**
Pre-harvest drop (as % no. berries)	3·9	9·7 **	7·8**
Total drop (as % no. of flowers)	33·7	36·1	48·0**
Mean berry weight (mg.)	553	541	606*
Mean crop per bush (kg.)	2·02	1·84	1·78
Westwick Choice (Harvested July 21)			
Date of spraying	—	April 13	May 17
Early drop (as % no. of flowers)	41·1	57·0**	49·0**
Pre-harvest drop (as % no. berries)	3·5	7·7**	12·0**
Total drop (as % no. of flowers)	43·2	60·2**	55·1**
Mean berry weight (mg.)	608	597	654*
Mean crop per bush (kg.)	2·18	1·61**	2·14

* Difference from control significant at 5%.
** Difference from control significant at 1%.

occasions. The early spray was applied just before the opening of the flowers, and the late spray after an interval of about three weeks, which was just ahead of the time when the heaviest drop was expected. On the Seabrook's plantation there were 6 blocks, each containing 3 plots of 40 bushes ; and on the Westwick plantation 9 blocks each containing 3 plots of 20 bushes. The plots were separated by guard rows, and spray drift from one plot to another was minimised by erecting screens of waterproof fabric during spraying. Abnormally small or large bushes were not included in the experiment, and a correction was made for these, and for missing bushes, in the final analysis. The total number of excluded or missing bushes was only 11 out of 720 for Seabrook's Black, and 74 out of 540 for Westwick Choice. At weekly intervals throughout the season records were taken of berry drop by counting all the berries remaining on 30 tagged bushes in each treatment. At harvest the total crop weight from each plot was recorded, and random samples of 45 trusses (from Westwick Choice) and 60 trusses (from Seabrook's Black) were taken for more detailed analysis of fruit number and fruit weight. The crop weight records were analysed using an analysis of covariance technique with two separate calibrating variates, allowing independently for gaps in the plots and for rejected bushes. The significance of the fruit drop measurements was estimated by means of standard χ^2 tests. The results of this trial are summarized in Table I.

Results.

Seabrook's Black. Contrary to the indications of the 1952 pilot experiment, no significant reduction in " running off " was obtained following the application of NAA at the flower-bud stage. There was, on the other hand, a marked and highly significant increase in pre-harvest drop. This effect was general on all the sprayed bushes of both varieties, and is thought to be an indirect effect of a slight hastening of maturity induced by the growth-substance. Had the sprayed bushes been picked one week earlier than the controls, this increased loss of ripe berries could have been avoided and the total crop increased by 4 to 8%. In contrast to the early spray, which had little effect on " running off," the late spray on May 17th greatly increased it, and it was observed that most of this increased drop occurred over the course of a few days, one week after the spray application. However, this increased drop following the late spray was largely compensated by an increased growth of the berries that remained, so that the final crop weight was not significantly less than on the unsprayed controls.

Westwick Choice. In this variety both the early and the late application of NAA increased " running off." As in Seabrook's Black, the increased drop following the late spray was compensated by an increased berry weight, so that there was no overall effect on crop weight. No increase in berry weight was obtained following the early spray, however, so that the increased drop was here reflected in a marked reduction in total crop weight.

Discussion.

It would appear from this experiment that there is little prospect of being able to use naphthalene acetic acid sprays to reduce " running off " of black currants in the field. On the two varieties tested the treatment was either

without effect or it acted as a thinning spray, increasing rather than decreasing fruit drop. The results are thus essentially similar to those already obtained with apple, where naphthalene acetic acid sprays applied at any stage between full bloom and three weeks after petal-fall have been shown to increase the intensity of the early periods of fruit drop. In the apple the increased drop has been shown to result from seed abortion induced indirectly by the growth substance (1). In the black currant, as discussed in the previous paper of this series (3), " running off " is also associated with seed abortion, and it seems possible that the effect of naphthalene acetic acid in increasing the drop may, as in the apple, be brought about by some indirect effect leading to increased abortion of the developing ovules. These results therefore strengthen the belief, expressed in the previous paper, that " running off " of currants is a physiological drop comparable to the " June drop " of apples.

Summary.

(1) Naphthalene acetic acid at 10 p.p.m. was applied as a spray to bushes of Seabrook's Black and Westwick Choice either at the flower bud stage or just prior to the early drop, with the object of reducing " running off."
(2) Neither spray had the desired effect, both treatments increasing rather than decreasing fruit drop. Following the late spray the decreased set was compensated by an increased berry weight.
(3) Both sprays increased pre-harvest drop of berries, probably as a result of a slight hastening of maturity.

Acknowledgments.

The author is indebted to Mr. G. M. Clarke for advice on the lay-out of the plots and for the statistical analysis of the data, and to Mr. E. Catlow and Mr. P. Weaver for collection of the harvest records.

REFERENCES.

(1) Luckwill, L. C. (1953). Studies of fruit development in relation to plant hormones : II. The effect of naphthalene acetic acid on fruit set and fruit development in apples. *J. hort. Sci.*, **28,** 25–40.
(2) Teaotia, S. S. (1953). Studies on fruit development and fruit drop in black currants. *Ph.D. Thesis, University of Bristol,* 1953.
(3) Teaotia, S. S. and Luckwill, L. C. (1956). Fruit drop in black currants : Factors affecting " running-off." *Ann. Rep. Long Ashton Res. Sta.*, 1955, 64–74.

THE PROPAGATION OF GOOSEBERRIES: III. THE EFFECT OF TIME OF TAKING AND PLANTING HARDWOOD CUTTINGS AND THE INFLUENCE OF BUDS ON ROOTING.

By BARBARA A. RAKE.

Introduction.

Previous experiments have shown that the time of planting gooseberry hardwood cuttings is an important factor in rooting (1). In 1951 four planting times (August-November) were compared, using the varieties Leveller and Keepsake. With both varieties September gave better results than August or October : with Keepsake, however, November gave as satisfactory results as September for percentage rooting but not for vigour. In 1952, comparing only August and December plantings of Leveller, Keepsake and Careless, very much more vigorous plants resulted from the early date of planting, but the percentage rooting was the same for both months. There were also indications that the " heeling-in " for two months of cuttings taken in October gave better results than cuttings removed from the bush immediately prior to planting in December. Similar beneficial effects of storing cuttings between taking and planting have been observed with quince (2).

In 1952 the retention of buds on the underground portion of cuttings of Leveller and Keepsake was found to have two opposing effects according to the time of taking the cuttings. Cuttings taken in August and planted without being disbudded rooted significantly better than disbudded cuttings, whereas in December the converse was true (1).

In 1954–55 the effects of time of taking and time of planting were examined together at monthly intervals from August to February. The effect of the removal or retention of buds at these times was tested in a separate experiment.

Experimental.

Experiment 1 : *Time of taking and planting cuttings.*

This test was carried out on three varieties, Leveller, Keepsake and Careless. Batches of cuttings were taken, and prepared 10 in. long, removing all buds except four at the top, at monthly intervals from August until February inclusive. From each batch one lot was planted immediately and the rest were tied in bundles and heeled-in for planting out at monthly intervals. This resulted in 28 treatments for each variety, giving a total of 84 plots per block. Owing to the large quantities of material involved only two replicates were possible. Each plot consisted of a row of 12 cuttings planted 6 in. apart ; the two end cuttings in each plot were not recorded.

Owing to the unusually wet summer of 1954 soil moisture conditions at the different planting times were remarkably similar, those of August and September being comparable with plantings made until February, when conditions were much drier and more favourable.

Cuttings taken in August were prepared from shoots not fully ripened, and those of Leveller were still growing at the tips. Batches taken in September

and October had ripened off but still had firmly adhering leaves. By November most of the leaves had abscissed. Bud burst had commenced on the cuttings taken in February.

Experiment 2 : *Effect of bud removal.*

This was carried out to test the effect of the retention of buds on the underground portion of the cutting at different planting times. Batches of cuttings were taken at monthly intervals and planted immediately, half having all buds except the four terminal ones removed, the other half having all buds intact.

Three varieties (Leveller, Keepsake and Careless) were used, replicated three times over seven months, giving a total of 126 plots. The source of cuttings, condition of material and soil conditions at planting time were similar to those in Experiment 1. At the end of 1955 when growth had ceased, records were made of percentage rooting, and vigour was scored visually in categories 1-10. Angular transformation was carried out on the results before analysis of variance.

Results.

Experiment 1 : *Time of taking and planting cuttings.*

Table I shows the condition at planting time of the cuttings taken at monthly intervals and heeled-in for varying periods.

The extent of the callus ring was estimated in categories ranging from 0 (indicating no callus visible) to 4 (callus ring approximately 1·5 mm. wide). Although basal callusing is not necessary for rooting, and well rooted cuttings may result without any formation of callus over the basal cut, it appears from this Table that intensity of callus activity is associated with root formation both near the basal wound and on the stem above.

August prepared cuttings showed little activity at all periods after heeling-in, and after a prolonged period the bark and cortex disintegrated. September prepared cuttings, on the other hand, showed marked activity ; callus and root formation were abundant, and the bark remained healthy throughout the six month period. The October prepared cuttings were also highly active. Cuttings prepared later in the year were less active but some activity remained despite prolonged hard frosts and very low soil temperatures.

It is evident from these records and from the results for rooting (Fig. 1) that the cuttings taken in August 1954 were not in fact hardwood cuttings, and could not be expected to withstand the conditions to which they were subjected. Seasonal variation in rooting trials of hardwood cuttings is common, and is shown by comparing the present results with those of the 1952 trials, when satisfactory rooting was obtained for all varieties planted in August, even when lower buds were removed. It is evident that hardwood cuttings cannot be taken before the shoots have ceased growing and the wood is at least semi-ripened ; this stage varies from year to year and would be expected to occur relatively late after the very wet summer of 1954.

Table II gives results for individual treatments and varieties. As transformed figures were used for the analysis of variance significant differences cannot be given.

TABLE I.

CONDITION OF HEELED-IN CUTTINGS AT PLANTING TIME.

Month prepared	Variety	Month planted					
		Sept.	Oct.	Nov.	Dec.	Jan.	Feb.
Aug.	L	no change	no change	no change	Bark rotted. Callus 0	Bark rotted. Callus 0	Bark rotted. Callus 0
	K	no change	no change	Few basal roots. Callus 1	Bark rotted. Few basal roots. Callus 1	Bark rotted. Few basal roots. Callus 1	Bark rotted. Few basal roots. Callus 1
	C	no change	no change	no change	Bark rotted. Few basal and nodal roots. Callus 2	Bark rotted. Few basal and nodal roots. Callus 2	Bark rotted. Few basal and nodal roots. Callus 2
Sept.	L	—	no change	Few basal roots. Callus 2	Many basal and nodal roots. Callus 4	As Dec.	As Dec.
	K	—	no change	Few basal roots. Callus 2	Many basal and nodal roots. Callus 4	As Dec.	As Dec.
	C	—	no change	Few basal roots. Callus 0	Many basal and nodal roots. Callus 4	As Dec.	As Dec.
Oct.	L	—	—	Callus 2	Few basal roots. Callus 2	Many basal roots. Callus 4	Many basal roots. Callus 4
	K	—	—	Callus 1	Few basal roots. Callus 2	Few basal roots. Callus 2	Few basal roots. Callus 4
	C	—	—	Callus 2	Callus 2	Many basal roots. Callus 4	Many basal roots. Callus 4
Nov.	L	—	—	—	no change	Callus 1	Few basal roots. Callus 3
	K	—	—	—	no change	Callus 1	Callus 1
	C	—	—	—	no change	Callus 1	Callus 2
Dec.	L	—	—	—	—	Callus 1	Callus 1
	K	—	—	—	—	no change	Callus 1
	C	—	—	—	—	Callus 1	Callus 1
Jan.	L	—	—	—	—	—	Callus 1
	K	—	—	—	—	—	no change
	C	—	—	—	—	—	Callus 1

TABLE II.

PERCENTAGE ROOTING AND VIGOUR OF VARIETIES AT DIFFERENT PREPARATION AND PLANTING DATES.

Month prepared	Variety	Month planted													
		Aug.		Sept.		Oct.		Nov.		Dec.		Jan.		Feb.	
		R	V	R	V	R	V	R	V	R	V	R	V	R	V
Aug. ..	L	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	K	15	4·5	5	2·0	20	2·5	10	2·0	10	3·5	0	0	5	1·0
	C	55	5·5	50	7·0	35	9·0	0	0	35	4·5	10	2·5	5	4·5
Sept. ..	L			70	3·0	65	5·5	75	7·5	55	4·5	55	3·0	70	5·5
	K			60	5·5	60	4·5	60	4·0	50	6·5	60	6·5	75	8·0
	C			100	6·5	85	8·0	70	6·5	75	4·0	60	9·0	95	9·0
Oct. ..	L					90	7·5	90	9·0	90	5·5	75	3·5	95	8·0
	K					20	3·5	50	5·5	45	2·5	65	4·5	85	8·0
	C					100	8·5	95	8·5	100	8·5	95	6·5	90	8·0
Nov. ..	L							70	4·0	40	4·0	90	4·5	85	5·0
	K							80	6·0	80	6·0	60	4·5	80	6·5
	C							100	8·5	90	8·0	75	5·5	95	8·5
Dec. ..	L									80	4·5	70	4·5	75	5·5
	K									80	4·0	75	4·0	85	5·5
	C									100	8·0	100	7·5	95	7·5
Jan. ..	L											80	5·0	70	3·0
	K											70	4·0	90	5·0
	C											80	5·5	85	5·5
Feb. ..	L													80	3·5
	K													45	4·5
	C													70	4·0

R = Rooting (per cent.). V = Vigour (categories 0-10).

The rooting of individual varieties, as in previous years, again showed a seasonal difference, with Careless (73·0%) giving significantly better results than Leveller (56·0%) and Keepsake (51·4%) at the 0·01% level.

Fig. 1 shows the percentage rooting and vigour expressed as the mean of the three varieties. Cuttings taken in August lose their low capacity to root as time of planting is delayed. Cuttings taken in September have a much higher initial capacity to root but this appears to decrease until February, when the initial capacity is regained. Whether this factor is contained in the

cutting itself or is due to soil conditions was not determined, but all February-planted cuttings, with the exception of those taken in August and February, show a rise in rooting percentage and in vigour (January also excepted). This seems to indicate that time of preparation is important only to the extent that cuttings root better if they are taken when they are fully dormant (i.e. September–3rd week December), but that within this range planting conditions appear more important. The poor results for cuttings taken and planted in January and February were repeated in Experiment 2.

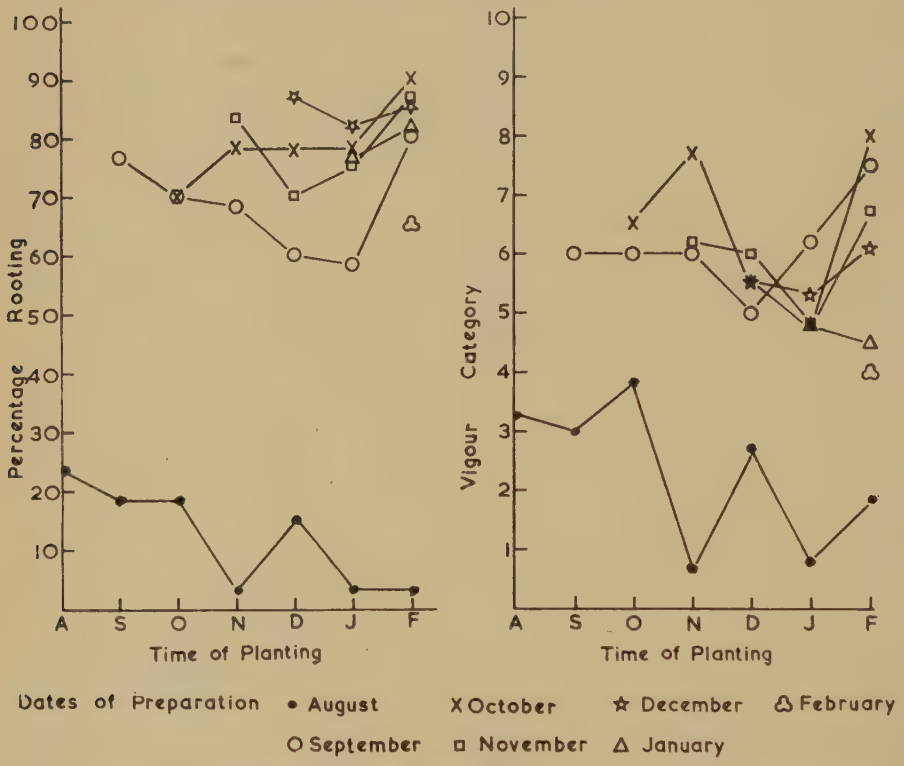


Fig. 1. (Expt. 1) Percentage rooting and vigour of gooseberry cuttings at different planting dates. Means of three varieties (6 plots).

In analysing individual treatments many differences were insignificant, and a good result for percentage take did not necessarily correspond with a good result for vigour. All August prepared cuttings gave results significantly lower than all other treatments at the 0·1 % level. The treatments giving significantly highest results for percentage rooting and vigour were : September prepared, February planted ; October prepared, February planted ; November prepared, February planted ; October prepared, November planted. The response of varieties to the treatments also differed slightly : Leveller appeared to become fit for cutting rather later and Careless rather earlier than Keepsake, but bearing in mind that the varieties came from different and widely separated locations this factor may be due to source rather than variety.

From the above results it would appear that " heeling-in " a cutting does not affect or delay its capacity to form roots and that this practice is preferable

to planting out in unfavourable conditions, provided that the cuttings themselves are in the correct condition at the time they are prepared. Owing to the formation of roots during heeling-in, care must be taken to prevent their breakage and/or drying out during planting.

Experiment 2 : *Effect of bud removal.*

In this experiment varietal differences and time (considered on the basis of immediate planting) showed the same trends as in Experiment 1, with a similar falling off for cuttings taken in January and February (Fig. 2).

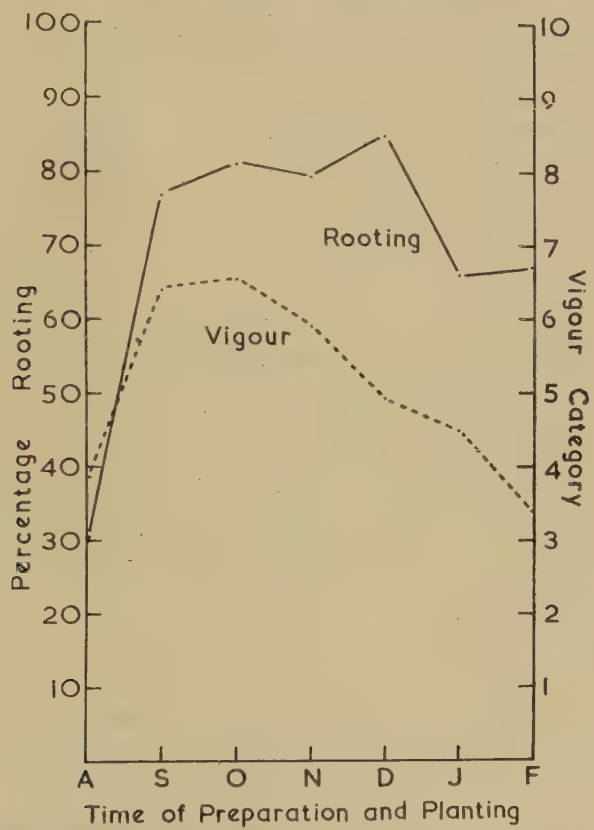


Fig. 2. (Expt. 2) Effect of time of planting (immediately after preparation) on rooting and vigour of gooseberry cuttings. Means of three varieties (18 plots).

Fig. 3 shows the general means for rooting of the three varieties planted at different times. It will be seen that cuttings with buds retained gave a significantly better result than those with buds removed in August (1% level) but significantly poorer results in December (0·1% level), January (5% level) and February (5% level). This effect is in line with previous results for the months of August and December only and appears to reflect the internal condition of the cutting, the period of no effect (Sept. Oct. Nov.) corresponding with the period of " true winter " dormancy of the buds, as opposed to

" summer " dormancy imposed by apical dominance (August) and " imposed " dormancy due to low temperatures (3rd and 4th week December, January and February). This result is not in agreement with Van der Lek's findings that the retention of dormant buds on poplar cuttings checked root development, the check diminishing as the dormant period approached its end (3). On the other hand, the effect may be bound up with the time of formation of flower primordia within the bud, but this has still to be investigated. In Leveller no flower primordia were found in the buds up to the last week in August. Buds examined in December contained fully formed flowers.

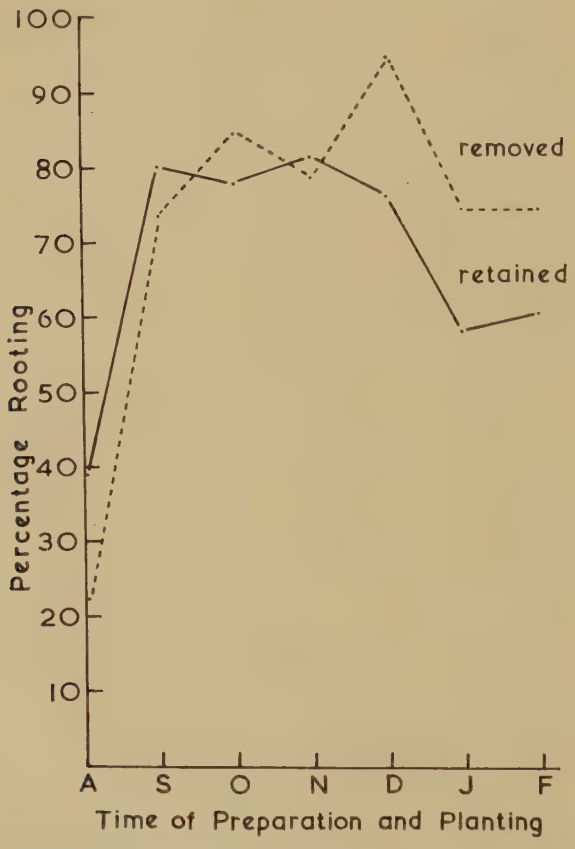


Fig. 3. (Expt. 2) Effect of retention of lower buds on percentage rooting of gooseberry cuttings taken at different times. Means of three varieties (9 plots).

Table III gives the percentage rooting for the three varieties, with and without buds, at each planting time. The bud effect shows up for all varieties in August but is significant only for Leveller; in December the effect is significant for all varieties (5% level). In January the effect is significant for Leveller and Careless (5% level) but not noticeable for Keepsake, while in February the effect is noticeably but not significantly reversed for Keepsake, while Leveller and Careless still show significantly the same bud effects as for December and January.

TABLE III.

THE EFFECT OF BUD RETENTION ON PERCENTAGE ROOTING.

Month prepared and planted	Leveller		Keepsake		Careless	
	+buds	—buds	+buds	—buds	+buds	—buds
August	23·3	0·0	30·0	16·7	63·3	50·0
September ..	86·7	70·0	60·0	56·7	93·3	93·3
October	83·3	93·3	63·3	63·3	53·3	96·7
November.. ..	93·3	76·7	56·7	63·3	93·3	90·0
December.. ..	80·0	93·3	73·3	93·3	73·3	96·7
January	46·7	76·7	60·0	63·3	66·7	83·3
February	56·7	86·7	73·3	50·0	50·0	86·7

For significance levels see text.

Maximum vigour was shown by the September, October and November plantings, but there was no bud/time interaction.

This experiment confirms the findings of the previous one that during the period September–November the shoots are in the optimum state for taking cuttings. It also shows that removal of buds from cuttings taken during this period is not necessary, provided that the cuttings are dug up and the suckers trimmed after one year in the cutting bed. If for any reason propagation is delayed until December or later it is desirable to remove buds in the varieties Leveller and Careless before planting the cuttings.

Summary.

Two experiments on the rooting of hardwood gooseberry cuttings are described.

The following factors were investigated :—

(1) Time of taking cuttings.
(2) Time of planting cuttings.
(3) The effect of disbudding cuttings at different times.

Time of taking cuttings proved to be an important factor ; September, October and November gave the best results.

Heeling-in of cuttings taken at these dates and planted in February in good soil conditions gave better results than planting them immediately when soil conditions were unsuitable.

Buds retained on the lower part of the cutting had a beneficial effect on cuttings prepared and planted in August, no effect on cuttings prepared and planted in September, October and November, and a deleterious effect on cuttings prepared and planted in December, January and February.

Acknowledgments.

Thanks are due to the following growers for generous supplies of cutting material : Mr. J. Firkins (Careless), Mr. J. Partridge (Leveller) and Mr. E. L. B. Jenkins (Keepsake) ; to Mr. G. M. Clarke and Miss M. E. Holgate for carrying out the statistical analyses ; and to Mr. P. S. Weaver and the plantation staff for their assistance.

REFERENCES.

(1) Rake, B. A. (1954). The propagation of gooseberries : I. *Ann. Rep. Long Ashton Res. Sta.*, 1953, 79.

(2) Tukey, H. B. and Brase, K. (1953). Random Notes on Fruit Tree Propagation. *Bull. N.Y. St. Agric. Exp. Sta.* 657.

(3) Van der Lek, H. A. A. (1934). *Meded. Landb. Hoogesch.*, Wageningen, Deel 38.

THE USE OF IRON CHELATES FOR THE CONTROL OF LIME-INDUCED CHLOROSIS IN FRUIT: PROGRESS REPORT II.

By C. BOULD.

Introduction.

Considerable progress has been made in the control of lime-induced chlorosis in fruit since the last report was published (1). At that time preliminary experiments indicated that the iron chelate of ethylenediamine tetraacetic acid (Fe-EDTA) was not effective under alkaline soil conditions when used at rates that were effective under acid soil conditions. In 1954 further experiments were made on pear and plum growing in a highly calcareous soil of pH 7·8, using increased rates of application of Fe-EDTA as a soil dressing, and also varying rates of ferric diethylenetriamine pentaacetate (Fe-DTPA) and ferric N-hydroxyethylethylenediamine triacetate (Fe-HEEDTA). For pear the chelates were applied in solution around the trees (within a circle of 6 ft. diameter) and thoroughly watered-in. Soil treatments and foliage sprays were also applied to chlorotic plum trees, *var.* Burbank's Giant Prune. The soil dressings for plum were only lightly watered-in, a faulty procedure that probably accounted for the lack of response. The treatments, rates of application and effects on chlorosis have been summarized by Bould (2). Fe-EDTA and Fe-DTPA, at 8 oz. per tree, gave a complete control of chlorosis in pear and resulted in vigorous extension growth. These two chelates, at the above rate, supplied 32 and 23 g. of chelated iron respectively. The Fe-HEEDTA was applied at lower rates, due to limited supplies of this material, and there was no response to the highest soil dressing, which supplied only 7 g. of chelated iron per tree. There was no response by plums to soil dressings of Fe-EDTA or Fe-HEEDTA.

As a result of these field experiments, two main conclusions were reached : (1) that for a given area of treated soil the quantity of chelate applied must exceed a certain minimum amount before any response is shown to treatment, and (2) that the chelate must be thoroughly watered-in and transported fairly rapidly to the roots to be effective. Under the conditions previously described the minimum amount of Fe-DTPA required for the control of chlorosis in small pear trees was between 6 and 8 oz. per tree (17–23 g. Fe).

Subsequent laboratory studies at Long Ashton by Hill-Cottingham (3), and in the U.S.A. by Wallace *et al* (6), have helped to explain the anomalous behaviour of iron chelates under field conditions. It has been shown by these workers that two main reactions take place when iron chelates are mixed with calcareous clay soils. The first reaction, which is rapid, involves sorption of chelate molecules by the clay, and until the sorbing power of the clay, which is dependent on the soil type, has been satisfied, the movement of chelate in the soil is restricted. The second reaction, which is slower but of longer duration, involves the replacement, and subsequent precipitation, of iron from the chelate molecule. It is thought that calcium is the principal ion causing replacement in calcareous soils. This second reaction explains the lack of response to surface soil dressings of iron chelates when they are not thoroughly

TABLE I.

SOIL TREATMENTS, METHOD AND TIME OF APPLICATION.
(All treatments were applied within a circle 6 ft. in diameter).

Treatments	Weight of chelate applied (oz.)	Fe content (g.)	Method of application	Volume of water used (gal.)	Time of application
Fe-chelates of:—					
* N-hydroxyethylethylenediamine triacetic acid (HEEDTA) absorbed on vermiculite	24	20	surface dressing—watered-in	—	11.5.55
Liquid-HEEDTA	14	20	,, ,, ,,	—	,,
,, ,,	7	10	sub-soil injection	1	13.5.55
,, ,,	10½	15	,, ,,	1½	,,
,, ,,	14	20	,, ,,	2	,,
** Diethylenetriamine pentaacetic acid (DTPA)	4	10	,, ,,	1	20.5.55
,, ,, ,,	6	15	,, ,,	1½	,,
,, ,, ,,	8	20	,, ,,	2	,,
Lignin sulphonate (liquid)	17½	20	surface dressing—watered-in	—	11.5.55
,, ,, ,,	26¼	30	,, ,, ,,	—	,,
,, ,, ,,	35	40	,, ,, ,,	—	,,
Mimosa tannins (paste)		20	,, ,, ,,	—	30.6.55
,, ,, ,,		40	,, ,, ,,	—	,,
,, ,, ,,		20	sub-soil injection	2	,,
,, ,, ,,		40	,, ,,	2	,,
Myrobalan tannins ,,		20	surface dressing—watered-in	—	,,
,, ,, ,,		40	,, ,, ,,	—	,,
,, ,, ,,		20	sub-soil injection	2	,,
,, ,, ,,		40	,, ,,	2	,,

Commercial names : *= Ferro Versenol ; **= Chel 330 Fe.

watered-in. Light watering merely mixes the chelate with the surface soil and, unless heavy rain follows within a few days, most of the iron may be replaced before the chelate reaches the plant roots.

The partial elucidation of the behaviour of iron chelates in calcareous clay soils has intensified the search for other chelating agents that are water-soluble, stable under alkaline soil conditions, not sorbed to any extent by clay, yet capable of being absorbed by plant roots in the molecular form and of releasing iron within the plant. Wallace *et al* (*loc. cit.*) and Holmes and Brown (4) have recently described experiments with two new chelating agents that show promise. They are cyclohexane-1, 2-diaminotetraacetic acid (CDTA) and an aromatic polyaminocarboxylic acid (formula not available) known as 138. The iron chelates of both these compounds show greater stability in calcareous soils than any other chelates so far tested, and, in addition, Fe-138 is not sorbed to any appreciable extent by clay.

Because of the relative instability of iron chelates in calcareous soils, and the necessity for heavy watering to secure a successful response, a procedure that is not economic under orchard conditions, increasing attention is being paid to foliar sprays.

An account is here given of the field work on iron chelates carried out during 1955 ; the laboratory investigation will be described elsewhere. The field experiments were designed : (1) to compare the relative effectiveness of several iron chelates when applied as soil dressings ; (2) to determine whether sub-soil injection of iron chelate solutions would give a better control of chlorosis than surface applications : and (3) to test a number of new iron chelates as foliage sprays.

Experimental.

The experiments were carried out at site B (see Progress Report I) on a highly calcareous soil, using very chlorotic pears, *vars.* Conference and Fertility on quince rootstocks, as the test crop.

Soil treatments.

Details of the iron chelates used, together with methods and times of application, are given in Table I.

Two methods of application were tried : (1) surface distribution over a defined area followed immediately by thorough watering with a hose pipe ; and (2) sub-soil injection of aqueous solutions by means of a ' Mapic ' soil injector (5). In this method the requisite amount of chelate was dissolved in 1 to 2 gal. water and injected at about 15″ below the soil surface, using 6 to 8 injection points scattered at random around the tree within a circle of 6 ft. diameter.

Foliage sprays.

Previous experiments had shown that pears are relatively sensitive to damage from iron chelate sprays, particularly at concentrations exceeding 0·1%. In the present work two concentrations only were used, 0·05 and 0·1%, with the addition of 0·025% di-octyl sodium sulphosuccinate as wetting agent. This wetter was compatible with all the chelates used and resulted in a uniform

wetting of the leaves. An attempt was made to spray both upper and lower leaf surfaces. Details of treatments and times of application are given in Table II.

TABLE II.

FOLIAGE SPRAY TREATMENTS, CONCENTRATION AND TIME OF APPLICATION.

Treatments	Concentration and date of application			
	22.6.55	30.6.55	8.7.55	22.7.55
Iron chelates of :—				
Ethylenediamine tetraacetic acid (EDTA-Na)	0·05%	—	0·1%	0·1%
„ „ „ (EDTA-Na_2)	0·05%	—	0·1%	0·1%
Diethylenetriamine pentaacetic acid (DTPA)	0·05%	—	0·1%	0·1%
Nitrilo acetic acid	0·05%	—	0·1%	0·1%
Cyclohexylenediamine tetraacetic acid (CDTA)	0·05%	—	0·1%	—
Saccharic acid	—	0·05%	0·1%	0·1%
Gluconic acid	—	0·05%	0·1%	0·1%
bis-Hydroxy-ethylglycine	—	0·05%	0·1%	0·1%
Hydroxyethylethylenediamine triacetic acid (HEEDTA)	—	0·05%	0·1%	0·1%

Results.

Soil Treatments.

Fe-HEEDTA. Aqueous solutions of this chelate, when applied as surface dressings to small pear trees, with subsequent heavy watering, gave a seasonal control of chlorosis with treatments supplying 20 g. of chelated iron per tree. Sub-soil injection was equally effective when the amount of iron was reduced to 10 g. per tree. The response to treatments using chelate absorbed on vermiculite was slower and this material was not so effective as aqueous solutions supplying equivalent amounts of iron. Treated trees made vigorous extension growth, and produced normal-coloured fruits. Control trees showed very severe chlorosis and necrosis by the end of July, made little or no extension growth and produced small crops of highly coloured fruit.

Fe-DTPA. When used at equivalent iron rates the responses to sub-soil injection of this chelate were not so good as for Fe-HEEDTA. Treatments supplying 20 g. of chelated iron per tree controlled all but a trace of chlorosis. Extension growth was good and fruits were normal in size and colour. Sub-soil injection of this chelate did not appear to be any more effective than surface application followed by heavy watering.

Residual effects from 1954 soil treatments. Trees treated in the previous year with 8 oz. of Fe-EDTA or Fe-DTPA, applied as surface dressings followed by heavy watering, maintained their vigour during 1955 and showed only a trace of chlorosis towards the end of the growing season.

Fe-lignin sulphonate. There was no response to surface dressings of this chelate over the range 20–40 g. iron per tree.

Fe-tannates. There was no response to surface dressings, or sub-soil injections, over the range 20–40 g. iron per tree.

Foliage Sprays.

Fe-EDTA. The first spray at 0·05% concentration caused no damage and resulted in a slight patchy recovery. Subsequent sprays at 0·1% concentration caused slight marginal and tip scorch of the leaves. By the middle of August sprayed trees had made a 70–80% recovery from chlorosis, as judged by leaf colour.

Fe-DTPA. This chelate at 0·1% caused slight marginal and tip scorch. The response was slightly poorer than with Fe-EDTA.

Fe-HEEDTA. There was no leaf damage at either concentration and control of chlorosis was good. In mid-August sprayed trees showed only a trace of chlorosis.

Fe-CDTA. Owing to very limited supplies only two sprays were applied (see Table II). No leaf damage was caused and the control of chlorosis was good. Absorption and/or translocation of iron from this chelate appeared to be greater than that from any other chelate tested.

Fe-saccharate, Fe-gluconate, Fe-hydroxyethylglycine. The response to these chelates was poor. No leaf scorch was caused at either concentration.

Fe-nitrilo acetate. This compound, at 0·1% concentration, caused moderate damage to leaves. The control of chlorosis was patchy.

Discussion.

It would appear from the results of these field experiments on small, severely chlorotic pear trees that at least a temporary control of lime-induced chlorosis can be obtained by soil dressings of some iron chelates if they are transported to the roots within a comparatively short time. This can be done by heavy watering, or by sub-soil injection of aqueous solutions. Laboratory experiments have shown that a fairly rapid loss of iron takes place from the chelate molecule when iron chelates are left in contact with calcareous clay soils. Furthermore, soils containing clay sorb a certain quantity of chelate, thereby restricting penetration, and this amount has to be exceeded before surface applications can be effective. Therefore, control of lime-induced chlorosis by soil dressings, with the iron chelates at present available, is still an uncertain method and is possibly uneconomic under orchard conditions. Sub-soil injection of aqueous solutions of Fe-HEEDTA reduces the amount of chelate required to control chlorosis, but this economy is off-set by the cost of injection.

Foliage sprays are less expensive and three to four foliar applications, at concentrations not exceeding 0·1% (plus a wetting agent), will give a seasonal commercial control of chlorosis in deciduous fruits. The translocation of foliar-applied iron chelate (via the phloem) is very much slower, and limited in range, than that absorbed by the roots and translocated via the xylem. For this reason foliage sprays need to be repeated at intervals so as to treat newly-formed leaves. Moreover, to be effective, the sprays must thoroughly wet the

leaves ; they should therefore be applied prior to fungicidal or insecticidal sprays : otherwise the deposits of these materials may be washed off.

Although some of the iron chelates so far tested are effective in controlling lime-induced chlorosis if used in the correct manner, they are not sufficiently stable in alkaline soils for general use, and a search must be made for other chelating agents that are reasonably cheap, not sorbed to any appreciable extent by clay, and are chemically and biologically stable under soil conditions that induce iron chlorosis.

Acknowledgments.

I wish to express my thanks to Mr. C. A. Barnard for permission to use his orchard, to the Geigy Co. Ltd., Manchester, for supplying most of the synthetic iron chelates, to F. W. Berk and Co. Ltd., London, for the Ferro Versenol (Fe-HEEDTA), to the Forestal Land, Timber and Railways Co. Ltd., for the tannins, and to Norman Evans and Rais Ltd., for the lignin sulphonate.

REFERENCES.

(1) Bould, C. (1954). *Ann. Rep. Long Ashton Res. Sta.*, 1953, 91.
(2) Bould, C. (1955). *Nature*, **175**, 90.
(3) Hill-Cottingham, D. G. and Lloyd-Jones, C. P. *Plant and Soil* (in the press).
(4) Holmes, R. S. and Brown, J. C. (1955). *Soil Sci.*, **80**, 167.
(5) Tolhurst, J. A. H. and Bould, C. (1950). *Ann. Rep. Long Ashton Res. Sta.*, 1949, 40.
(6) Wallace, A., Mueller, R. T., Lunt, O. R., Ashcroft, R. T. and Shannon, L. M. (1955). *Soil Sci.*, **80**, 101.

FOLIAR ANALYSIS IN RELATION TO BLACK CURRANT NUTRITION: I. INVESTIGATIONS ON LEAF DISC SAMPLING.

By C. BOULD and E. G. BRADFIELD.

Introduction.

Previous work on black currants at Long Ashton (1, 2) showed that a relationship existed between the nitrogen status of leaves, taken from the mid-stem position of extension shoots in early July, and the yield of fruit in the following season. Later work on leaf analysis (3) showed that there was a considerable variation in the major nutrient status of leaf samples due to position and time of sampling. For total nitrogen and phosphorus, and to a lesser extent total potassium, variation due to position was least from the middle of June until fruit ripening, and during this period the concentration of nitrogen and phosphorus in leaves from the mid-stem position remained fairly constant.

In 1954 a new field experiment was started with the object of making a more detailed study of the relationship between leaf nutrient status, shoot growth and yield of fruit of black currants. The layout is a well replicated randomized block with fertilizer treatments ($3 \times 2 \times 2$ N.P.K. factorial) applied to widely-spaced single-bush plots. The use of single-bush plots necessitated a modification in the method of leaf sampling, since the removal of a number of leaves from a young bush, on more than one occasion during the season, would probably have an adverse effect on shoot growth. A preliminary sampling experiment was therefore carried out to determine: (1) whether a disc sample taken from a particular part of the leaf was representative of the whole; and (2) whether the removal of one or more discs from a leaf affected the total nitrogen and potassium concentration of subsequent discs taken from the same leaf.

Experimental.

Experiment 1.

Sampling method. On 21.6.55 ten non-fruiting bushes of the variety Mendip Cross (cut to ground level in the previous winter and given the same manurial treatment) were chosen from a uniform row of plants; ten shoots per bush were selected, and from each shoot the fifth leaf from the unopened terminal cluster was removed. The ten leaves from each bush constituted one sample. For each sample, discs were punched from individual leaves (as illustrated in Fig. 1) with a cork borer of 13·5 mm. diameter, discs from similar positions being bulked to form sub-samples A — D. The mean area of leaf removed in one disc was approximately 1/50th of the total, and the mean dry weight of a 10 disc sub-sample was 72 mg. After removal of the discs the leaves-plus-petioles from each bush were bulked.

Chemical analysis. Total nitrogen and potassium were determined in each sub-sample and in the residual leaves, nitrogen by a micro Kjeldahl procedure and potassium by the flame photometer. The results, together with tests of significance ("t" test), are given in Table I.

Fig. 1. Positions of disc sampling (Experiment 1).

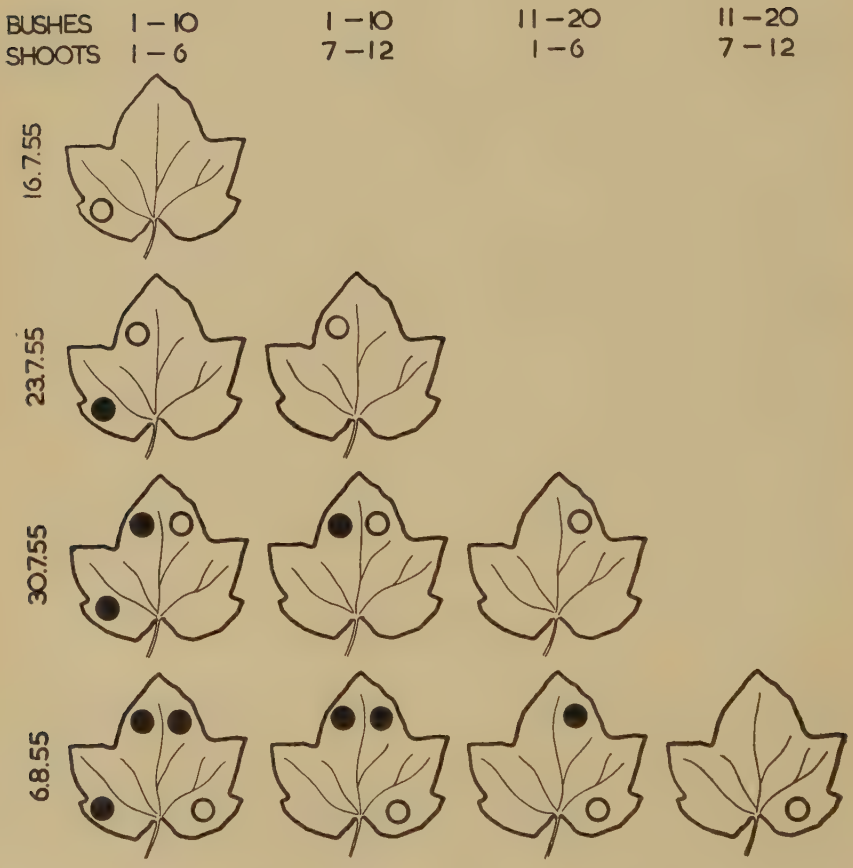


Fig. 2. Position and date of disc sampling (Experiment 2).
Black circles represent discs removed at previous samplings.

Experiment 2.

Sampling. Twelve shoots were selected on each of 20 bushes (var. Mendip Cross) and the fifth leaf from the unopened terminal cluster labelled. Disc sampling was carried out as described below and illustrated in Fig. 2. The black circles in Fig. 2 represent discs removed at previous samplings.

Sampling date	Bushes 1–10		Bushes 11–20		Effect studied. Previous removal of
	Shoots 1–6 Disc position	Shoots 7–12 Disc position	Shoots 1–6 Disc position	Shoots 7–12 Disc position	
16.7.55 ..	A	—	—	—	—
23.7.55 ..	B	B	—	—	A on B.
30.7.55 ..	C	C	C	—	A + B on C; B on C.
6.8.55 ..	D	D	D	D	A + B + C on D; B + C on D; C on D.

Each disc sub-sample therefore consisted of 6 discs, the mean dry weight being 55 mg. The samples were analysed as in Experiment 1 ; the results are given in Table II.

Results.

The results of Experiment 1, comparing whole leaf with disc sampling, are shown in Table I. Nitrogen is not significantly different whereas potassium in disc samples is significantly lower than in whole leaf plus petiole (at 1% level).

TABLE I.

COMPARISON OF WHOLE LEAF WITH DISC SAMPLING.
Sampled 21.6.55.
(Data obtained from mean of 10 bushes and expressed on dry weight basis).

Sample	% N	S.E. of mean	Significance of difference from whole leaf	% K	S.E. of mean	Significance of difference from whole leaf
Whole leaf ..	3·40	0·018	—	2·43	0·058	—
Disc A	3·44	0·025	N.S.	2·16	0·036	Sig. at 1%
Disc B	3·43		N.S.	2·14		Sig. at 1%
Disc C	3·43		N.S.	2·12		Sig. at 1%
Disc D	3·45		Sig. at 5% (just)	2·20		Sig. at 1%

The results of Experiment 2, showing the effects of previous disc sampling of leaves on the nitrogen and potassium concentration of subsequent samples are given in Table II. There is no significant effect on nitrogen concentration and with one exception, that of A on B, there is no effect on potassium concentration.

Discussion.

It would appear from the results of Experiment 1 that disc samples taken at any of the positions A to D, shown in Fig. 1, are representative of the whole leaf so far as total nitrogen is concerned. For total potassium, differences due to position within the leaf are not significant, but the disc samples are significantly lower in potassium than the whole leaf plus petiole. This may be due to the petiole having a higher potassium status than the lamina, although no data are available from the experiment to support this suggestion.

TABLE II.

EFFECTS OF PREVIOUS DISC SAMPLING OF LEAVES ON THE NITROGEN AND POTASSIUM CONTENT OF SUBSEQUENT SAMPLES.
(Data obtained from mean of 10 bushes and expressed on dry weight basis).

Date of sampling	16.7.55	23.7.55		30.7.55			6.8.55			
Position of disc on leaf	A	B		C			D			
Key to bush and shoot numbers (see below)	1	1	2	1	2	3	1	2	3	4
% N	3·42	3·22	3·19	3·11	3·23	3·15	3·03	3·04	2·98	3·01
S.E. of mean	0·025	0·018	0·028	0·021	0·037	0·025	0·022	0·017	0·022	0·012
Significance of difference between previously punched and unpunched leaves	—	N.S.	—	N.S.	N.S.	—	N.S.	N.S.	N.S.	—
% K	2·67	2·54	2·42	2·34	2·40	2·32	2·41	2·47	2·46	2·38
S.E. of mean	0·028	0·016	0·027	0·041	0·038	0·049	0·040	0·034	0·061	0·055
Significance of difference between previously punched and unpunched leaves	—	Sig. at 1%	—	N.S.	N.S.	—	N.S.	N.S.	N.S.	—

Key :— (1) Bushes 1 — 10. Shoots 1 — 6.
(2) Bushes 1 — 10. Shoots 7 — 12.
(3) Bushes 11 — 20. Shoots 1 — 6.
(4) Bushes 11 — 20. Shoots 7 — 12.

The second experiment shows that the removal of one to three discs from the same leaf, during the period 16th July to 6th August, had no significant effect on the nitrogen status of that leaf. It should be emphasized, however, that this result applies only to the particular period studied and that a different effect might be observed if samples were taken earlier in the year when the nitrogen status of leaves is changing rapidly.

With one exception no effects on the potassium status of the leaf due to previous disc sampling were observed : the removal of discs from the A position had a statistically significant effect on the potassium status of discs taken one week later from the B position. It is doubtful whether this is a real difference since the effects of A + B on C, and A + B + C on D are not significant.

It appears from these experiments that disc samples can be used instead of whole leaves for nitrogen determinations where the amount of material available is limited. Further experimental work will be needed to study : (1) the relative potassium status of petiole and lamina ; and (2) the reliability of disc sampling methods at other periods during the growing season.

Summary.

A comparison of disc versus whole leaf-plus-petiole samples of black currant leaves showed no significant differences in nitrogen status, but the disc

samples were significantly lower in potassium. With one exception the removal of one to three discs from the same leaf had no effect on the nitrogen or potassium status of subsequent disc samples.

Acknowledgment.

We wish to express our thanks to Mr. G. M. Clarke for statistical advice.

REFERENCES.

(1) Bould, C. and Catlow, E. (1948). A manurial experiment on black currants. Progress report II. *Ann. Rep. Long Ashton Res. Sta.*, 1947, 52–8.
(2) ——, —— (1951). Progress report III. *Ann. Rep. Long Ashton Res. Sta.*, 1950, 49–54.
(3) Bould, C. (1955). Seasonal changes in the major nutrients of black currant leaves. *J. hort. Sci.*, **30,** 188–196.

SPRAY APPLICATION PROBLEMS: XXI. THE RAPID ASSESSMENT OF DEPOSITS OF COPPER FUNGICIDES.

By J. T. MARTIN.

Introduction.

In collaborative work between the Plant Pathology Sections at Long Ashton, considerable importance is attached to the use of chemical methods for the determination of insecticide and fungicide deposits in measuring the effects of sprays. In the field assessment of an insecticide, for example, observations on the degree of insect control achieved have greater value and meaning when they are related to the level of deposit responsible for the biological effect obtained. Work on the development of spraying machines is assisted considerably if the amount and distribution of spray material deposited on the target are determined. Investigations of the relative values of different formulations of spray materials and of the effect of the nature of the treated surface upon their retention also call for methods for assessing deposits. Another aspect of importance, when persistent chemicals that are toxic to humans are used, is the measurement of the degree of contamination of crops at harvest time. For these reasons, attention has been given to the development of suitable analytical methods (see also pp. 103 and 106). So far as possible, the methods should be rapid and simple in operation since large numbers of samples may have to be examined.

Analysis of Deposits.

An earlier paper (1) dealt with the levels and distribution of copper deposits obtained in field trials using large and small volume spraying methods. Analyses were made of whole potato leaves, which were ashed and the copper determined by a standard method using sodium diethyldithiocarbamate for the colour development. Williams and Morgan (2) have recently described the use of a new reagent, bis-*cyclo*hexanone oxalyldihydrazone, C_6H_{10}:N.NH.CO.CO.-NH.N:C_6H_{10}, following nitric-perchloric acid digestion of plant material. In work on spray damage with copper sulphate, Allen and Llewelyn (3) have also used this reagent and describe a rapid method, involving preliminary ashing of the material, for the determination of copper sulphate in amounts up to 2·5 mg. on whole leaves.

Investigations of the distribution of copper on leaf surfaces required a method that would determine deposits on small areas. Since *surface* deposits only were involved, and in order to reduce the number of operations to a minimum, attempts were made to recover the copper directly from the treated surface. It was found that the dihydrazone reagent, in a slightly ammoniacal medium, would remove the copper, present as Bordeaux or Burgundy mixture, cuprous oxide or copper oxychloride, from fresh or wilted bean, laurel or *Coleus* leaves. A blue colour, of varying intensity according to the amount of copper present, was formed in the washing solution and, after the removal of this, no more copper could be detected by further addition of the reagent.

Tests with Burgundy mixture, pipetted in diluted form on leaves and allowed to dry, gave recoveries over the range 2·5–50 μg. Cu that were sufficiently quantitative (in view of the difficulty in applying accurately a sedimenting suspension) to justify the method. When the method was applied to the analysis of small discs of leaves sprayed with Bordeaux mixture formulations and afterwards dried to a brittle form, at the higher levels of deposit the discs retained quantities of up to 2 μg./sq. cm. of copper (determined by ashing and standard dithiocarbamate treatment). Although some of this copper may have been within the leaf tissue, it has been thought advisable to incorporate an acid wash to ensure the removal of most of the surface copper before the development of the colour.

Method of Analysis.

Reagents.

Sulphuric acid : Approximately N. Dilute 14 ml. of concentrated acid with water to 500 ml.

Ammoniacal ammonium citrate : To 50 ml. of 10 per cent. ammonium citrate solution add 60 ml. of 0·880 ammonia and dilute to 500 ml. with water. Adjust the strength of the ammonia so that 10 ml. of the acid are neutralized by 5 ml. of the ammonia solution using neutral red as indicator.

Bis-*cyclo*hexanone oxalyldihydrazone reagent: 0·1 per cent. in 50:50 ethyl alcohol-water. Dissolve by warming. Keep in a dark bottle and renew frequently. Before use dilute to 2·5 volumes with water.

Teepol wetter : Diluted 1 in 4 with water.

Method.

Place the leaf sample in a conical flask and add 10 ml. of N sulphuric acid and 1 spot of wetter. Swirl and allow to stand 10 minutes. Add 5 ml. of the ammoniacal ammonium citrate solution and 5 ml. of the dihydrazone reagent, with swirling. If a blue colour fails to develop, make certain that this is not due to acidity by adding a few drops of the ammonia solution. Allow to stand for 45 minutes, with occasional agitation. Measure the optical density of the colour spectrophotometrically or compare with a standard coloured solution, prepared from copper sulphate, in a Duboscq-type colorimeter.

The colour requires 30–45 minutes to develop and is then stable for some hours. For spectrophotometric measurement, we have used the Unicam S.P. 600 instrument set at 595 mμ, and a 1 cm. cell. The linear relationship, over the range 0–50 μg., between copper (as sulphate) and optical density is shown in Fig. 1. Automatic pipettes have been used for convenience in handling large numbers of samples.

The Distribution of Copper on Leaves.

Important characteristics of a deposit of a protective fungicide are the mean level of the deposit, its regularity of distribution and the proportion of the plant surface it covers. Good distribution does not necessarily mean good

coverage ; a leaf surface may be completely covered by a deposit that is unevenly distributed, or a considerable proportion of the surface may be untouched by a deposit that is evenly distributed, such as may result from the impaction of droplets in small-volume spraying.

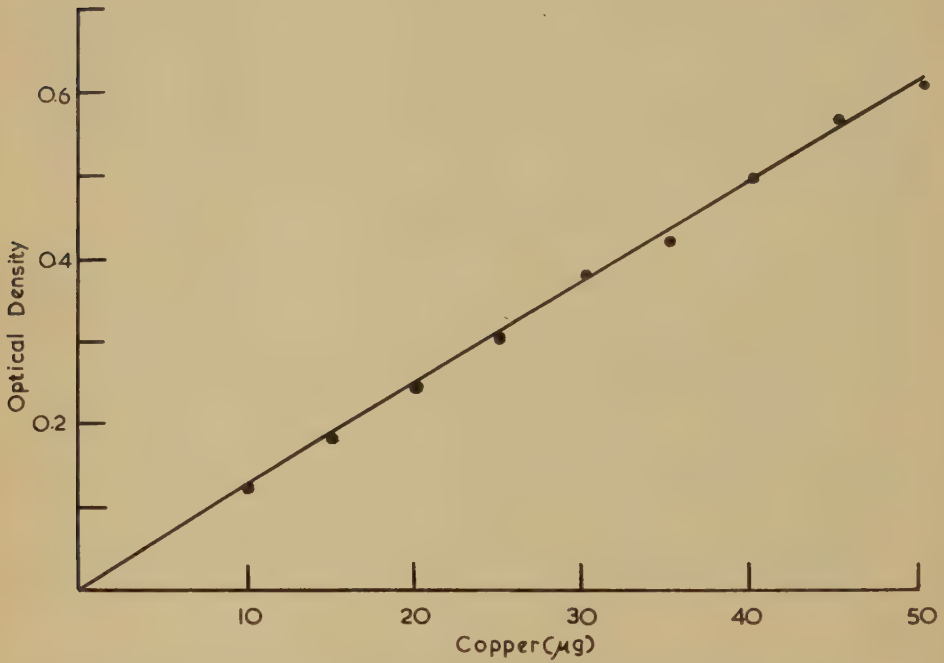


Fig. 1. Calibration curve for determination of copper.

Variation in the intensity of deposition over a treated surface may be determined by the analysis of discrete areas of the surface, and from the data obtained the mean level of deposit (μ) and its standard error (σ) may be found. In assessing the efficiency of distribution achieved by a treatment, the standard error of the mean deposit considered alone is of little help, since it will usually increase in magnitude as the size of deposit increases. Whatever the level of deposit, a measure of the efficiency of distribution may be obtained by calculation of the percentage coefficient of variation, V, where

$$V = \frac{100\ \sigma}{\mu}$$

For perfect distribution, $V = 0$; the less efficient the distribution, the greater will be the value of V.

As an example of its application, the analytical method described above was used to determine the levels and distribution of copper deposits on banana leaves subjected to different treatments. Discs (3 sq. cm. in area) were taken at random from dried leaves using the sampling tool described by Morgan (4). Ten discs from each treatment were analysed individually. After the measurement of the optical density of the colour, the discs from each treatment were

combined, washed with distilled water, and residual copper determined by ashing and the dithiocarbamate method.

Measurement of the amount of constriction of the leaf during drying permitted the expression of results as μg. of Cu per sq. cm. of fresh leaf surface. The results are given in Table I.

TABLE I.

ANALYSIS OF BANANA LEAVES FOR COPPER DEPOSITS.

Treatment	Range	Mean (μ)	S.E. (σ)	Residual	Percentage coeff. of variation, V
1	0·7– 1·8	0·97	0·37	0·3	38
2	0·0– 1·2	0·78	0·34	0·2	44
3	4·8– 9·1	6·84	1·17	0·3	17
6	6·9–10·8	8·96	1·41	0·2	16
8	6·8–11·7	8·64	1·85	0·6	21
15	9·0–24·0	18·0	4·9	0·4	27
23	5·2–12·7	7·94	2·51	0·4	32

Check analyses were made on leaf samples from treatments 6 and 23 by ashing and colour formation by the standard dithiocarbamate method. The values obtained (8·3 μg Cu sq. cm. of fresh leaf in each case) were of the same order as those given by the dihydrazone method.

The levels of deposits from the different treatments vary appreciably; these results will be discussed when the work is fully published later. Judged from the values of V, treatments 3 and 6 have given the most efficient distributions, closely followed by treatment 8.

It should be noted that the efficiency ratings obtained are influenced by the sampling procedure used. In comparisons of the efficiencies of distribution shown by treatments in separate experiments, the number and size of the discs taken for analysis should therefore be kept constant. The method represents an attempt to put the assessment of the distribution of deposits upon a quantitative basis. Information will be needed on the level of numerical rating that, for any given spray material and purpose, will represent a satisfactory distribution in practice.

Summary.

(1) A rapid method is described for the determination of surface deposits of copper fungicides on small areas of leaf tissue.

(2) A method is outlined for the quantitative assessment of the efficiencies of distribution of copper by different treatments.

Acknowledgments.

I am indebted to Mr. G. M. Clarke for helpful discussion and to Miss P. M. Jackson for analytical assistance.

REFERENCES.

(1) Morgan, N. G. and Martin, J. T. (1955). *Ann. Rep. Long Ashton Res. Sta.*, 1954, 97–105.
(2) Williams, T. R. and Morgan, R. R. T. (1954). *Chem. and Ind.*, 461.
(3) Allen, M. and Llewelyn, F. W. M. (1954). *Ann. Rep. East Malling Res. Sta.*, 1953, 180–181.
(4) Morgan, N. G. (1955). *Ann. Rep. Long Ashton Res. Sta.*, 1954, 171–172.
(5) Martin, J. T. (1953). *Ann. Rep. Long Ashton Res. Sta.*, 1952, 71–74.

SPRAY APPLICATION PROBLEMS: XXII. DETERMINATION OF CAPTAN DEPOSITS: II.

By J. T. MARTIN and J. A. PICKARD.

Introduction.

A colorimetric method for the determination of captan (N-trichloromethylmercapto-4-*cyclo*hexene-1,2-dicarboximide) deposits on apple and strawberry fruits and foliage was described in the 1954 Report (1). In this method the plant material was extracted with chloroform, the extract was passed through activated alumina, and a yellow colour, developed on heating with resorcinol, was measured in an acid-alcohol medium.

The method was not regarded as entirely satisfactory. Unduly high "blank" determinations were recorded, particularly in the examination of leaves, due to plant pigment not removed by the alumina. Further work has led to improvements of the method and these, with a description of the technique now used, are given in this report.

Method of Analysis.

We have found that the brand of charcoal designated Darco G-60* satisfactorily decolorizes benzene extracts of plant materials without loss of captan. We therefore prefer the Darco charcoal to alumina for the removal of interference by pigments, and benzene for the initial extraction. The solubility of captan in benzene is lower than in chloroform, but tests have shown that the extraction procedure described below is satisfactory.

The resorcinol is now added as a 15 per cent. solution in acetone, giving a more intimate admixture with the captan on the removal of the solvent. We have also adopted glacial acetic acid, in place of acid-alcohol, as the medium for the colour assessment, as recommended by the California Spray Chemical Corporation in 1955.

The particulars of the method now used are as follows :—

Reagents.

Benzene : Analytical Reagent grade, redistilled before use.
Resorcinol : Analytical Reagent grade, sublimed before use.
Acetic Acid Glacial : Analytical Reagent grade.
Darco Carbon G-60.
Resorcinol in acetone : 15 per cent. solution freshly prepared.

Extraction of fruits (strawberries).

Wash 100 g. of strawberry fruits in a beaker with 50 ml. and then with four successive portions of 25 ml. of warm benzene, decanting through a plug of cotton wool. Concentrate by gentle distillation and make up to 50 ml. volume.

* Obtainable from Darco Carbon Corporation, 42nd East Street, New York.

Extraction of leaves.

Extract 1 g. of powdered, air-dried leaves in a continuous percolator for 30 minutes, using 20–25 ml. of benzene. Make up to 50 ml. volume.

Method.

Take an aliquot to give a reading in the range 20–100 μg. of captan. If necessary, adjust the volume to 10–20 ml. by dilution with benzene or by concentration by distillation. Add 1 g. of Darco charcoal, rotate and allow to stand for 10–15 minutes. Filter into a boiling tube through a 9 cm. No. 54 Whatman filter paper and wash the filter with five portions of 3 ml. of benzene. Add to the filtrate 2 ml. of resorcinol solution and remove the solvent with a stream of dry air at 55°C. Place the tube in an oil bath at 135°C. for 15 minutes with occasional rotation. Remove from the bath and dissolve the residue, before solidification occurs, in 10 ml. of acetic acid. Filter if opalescent, and measure the optical density of the colour. We have used the Unicam SP 600 Spectrophotometer set at 425 mμ and a 1 cm. cell.

Read the μg. of captan from a standard line prepared from tests using captan crystallized (absolute ethyl alcohol) to m.p. 174·5°C. Ground-glass joints should be used throughout the method.

Captan Deposits on Strawberries.

An experiment was carried out in 1955, at the Luddington Experimental Horticulture Station, to compare the levels of deposit resulting from spray and dust application. The materials used were a dispersible powder containing 50 per cent. of captan and a dust containing 10 per cent. of captan. The dispersible powder was applied at a dosage of 0·25 per cent. captan at the rate of 200 gal. per acre per application, and the dust at the rate of 32 lb. per acre per application.

The treatments were commenced on 24 May, when half the blossoms were open, and were continued as follows :—

	Material.	*Applied.*			
A.	Dispersible powder	24 May	6 June	16 June	27 June
B.	,, ,, ...	24 May	6 June	16 June	—
C.	,, ,, ...	—	6 June	16 June	27 June
D.	,, ,, ...	24 May	—	16 June	—
E.	,, ,, ...	—	6 June	—	27 June
F.	Dust	24 May	6 June	16 June	27 June

Samples of the ripe fruits were taken on 12 and 16 July and, as in the 1954 experiments, separate analyses were made of the berries and calyces. The results are shown in Table I ; the values reported for the fruits are the mean determinations on three samples.

The four applications of the 10 per cent. dust failed to give a deposit on the fruits approaching that from the four applications of spray fluid containing 0·25 per cent. captan. The effect of the last application of the spray, made when the berries were reaching mature size, in raising the level of the deposit is clearly seen. The deposits on the fruits given by the dispersible powder formulation, however, were lower than those obtained in the 1954 trials and further work is needed.

TABLE I.

CAPTAN DEPOSITS (AS P.P.M.) ON STRAWBERRY FRUITS AND CALYCES.

	12 July		16 July	
	Fruits	Calyces	Fruits	Calyces
A	3·6	900	3·3	880
B	1·8	470	1·9	455
C	3·4	590	3·2	615
D	1·5	385	1·3	365
E	2·8	530	3·1	525
F	1·7	240	1·9	220
Control ..	0·4	10	0·3	10

Summary.

(1) Modifications of the method for the determination of captan deposits are described.

(2) In a preliminary trial, a comparison has been made of the levels of captan deposits resulting from dust and spray treatments of strawberries.

REFERENCE.

(1) MARTIN, J. T. and PICKARD, J. A. (1955). *Ann. Rep. Long Ashton Res. Sta.*, 1954, 83–89.

SPRAY APPLICATION PROBLEMS: XXIII. DETERMINATION OF DEPOSITS OF DDT, *GAMMA*-BHC AND CHLORBENSIDE.

By J. T. MARTIN and R. F. BATT.

Introduction.

Considerable progress has been made in recent years in the control of fruit tree pests by washes applied in the spring. DDT and *gamma*-BHC, applied pre-blossom, have given satisfactory results against aphids, apple sucker, caterpillars and capsids, and effective new acaricides, including chlorbenside (*p*-chlorobenzyl *p*-chlorophenyl sulphide), chlorfensone (*p*-chlorophenyl *p*-chlorobenzenesulphonate) and chlorobenzilate (ethyl *pp'*-dichlorobenzilate) are available for the control of Red Spider mites. Kearns and Morgan (1) and Bennett (2) have described the use of combined DDT-*gamma*-BHC emulsions of high wetting properties from bud-burst onwards. Other recent work at Long Ashton (3, and this Report, p. 125) has confirmed the value of chlorbenside and chlorfensone against the winter eggs of the Fruit Tree Red Spider mite when applied at 0·02 per cent. concentration at the pink bud stage of Worcester Pearmain. Kearns and Morgan (*loc. cit.*) pointed out that although their DDT-*gamma*-BHC wash was used at the low concentrations of 0·025 per cent. of DDT and 0·007 per cent. of *gamma*-BHC, there was a risk of increasing the liability of the trees to Red Spider attack. In more recent work (this Report, p. 32) they have incorporated chlorbenside (0·02 per cent.) in the spray fluid for application at the pink bud stage. Our interest in the behaviour and persistence of spray chemicals on plants and in the amounts necessary for efficient pest control has led us to a consideration of methods for the determination of DDT, *gamma*-BHC and chlorbenside occurring together in spray deposits.

Cranham and his colleagues (4), when first reporting the efficiency of chlorbenside as an acaricide, gave information on its physical and chemical properties. The compound shows a high lipoid solubility, an appreciable vapour pressure and is able to penetrate within leaves.

Chlorbenside (I) is easily oxidized to the sulphoxide (II) and then, under more vigorous conditions, to the sulphone (III).

I

II

III

Oxidation, at least to the sulphoxide, is believed to take place on the leaf surface. Although the oxidation products are not thought to be able to penetrate leaves to the same extent as the sulphide, they are as active as the sulphide against the eggs and immature forms of the Red Spider mite and are likely to account for the persistence of the material that has been observed in the field.

Our method for the determination of DDT deposits on plant materials has been described (5). It is similar in principle to the method of Schechter and his co-workers (6) but includes the passage of the initial extract through alumina (5 g.) to reduce wax and pigments. *Gamma*-BHC may be determined by the method of Schechter and Hornstein (7) in which the compound is reduced to benzene which is distilled, nitrated and a colour developed with potash in methyl ethyl ketone. A method for the determination of chlorbenside deposits has recently been published by Higgons and Kilbey (8) in which the compound after extraction is oxidised to the sulphone, nitrated and a purple colour formed with sodium methylate. Since the procedure closely follows that of the Schechter method for DDT, the presence of chlorbenside will interfere with the determination of DDT, and *vice versa*, as Higgons and Kilbey (8, 9) and earlier Cranham *et al* (*loc. cit.*) have pointed out.

Investigation of Methods.

The materials used were *pp'*-DDT, m.p. 108°C. ; *gamma*-BHC, m.p. 112–113°C. ; chlorbenside, its sulphoxide and sulphone. The chlorbenside and its derivatives were kindly sent to us by Messrs. Higgons and Kilbey. The chlorbenside, after recrystallization from 95 per cent. ethyl alcohol, melted at 72°C. and contained 26·8 per cent. of chlorine (theoretical 26·4 per cent.). The sulphoxide melted at 123–125°C. and the sulphone at 150°C.

Tests were made to ascertain to what extent interference would arise in the determination of each of the three constituents of the mixed spray deposit. *Gamma*-BHC in carbon tetrachloride solution was found to pass through the alumina column with the DDT, but did not interfere in its determination by our method. Neither did the presence of DDT influence the estimation of *gamma*-BHC by the reduction process. The determination of *gamma*-BHC was, however, upset by the presence of chlorbenside.

Chlorbenside, the sulphoxide and the sulphone, tested by direct nitration and our procedure for DDT, gave colours ; that from the sulphone being particularly intense. When the sulphide, sulphoxide and sulphone, each in a carbon tetrachloride extract of apple foliage, were passed through alumina before nitration, a slight pink colour was given by the sulphide but no colours resulted from the sulphoxide and sulphone, indicating the retention of these compounds by the alumina. The sulphone could be eluted from the column with chloroform and then gave a magenta colour with alcoholic potash after nitration. The sulphone, in carbon tetrachloride, was also retained on a column of silica gel, but this was less effective than the alumina in removing plant pigments.

It was clear that, in order to avoid interference in the determination of each of the three constituents of the spray deposit, it was necessary to separate the chlorbenside from the DDT and *gamma*-BHC, and that this could be done by retaining the chlorbenside, as sulphone, on alumina. Further experiments with the three compounds in leaf extract showed that the intensity of the magenta colour obtained from the sulphone held on the column could be used for the quantitative determination of the chlorbenside. The DDT and *gamma*-BHC were determined using aliquots of the carbon-tetrachloride solution passing through the alumina.

The deposits on leaves following the application of chlorbenside will consist of mixtures of varying proportions of the sulphide, the sulphoxide and possibly the sulphone, according to the age of the deposits. Tests were made to find an oxidizing agent and the experimental conditions necessary to convert the sulphide and sulphoxide to the sulphone without loss of the DDT or *gamma*-BHC. Hydrogen peroxide in glacial acetic acid was used, but potassium permanganate in diluted acetic acid was finally adopted. Comparative analyses, using leaf extracts, with the sulphide oxidized before passage through alumina and with equivalent amounts of the sulphone without pre-oxidation, gave agreement within the limits of experimental error.

An examination of the Schechter-Hornstein procedure for the determination of *gamma*-BHC was made by Mr. J. A. Pickard and will be fully reported later. Difficulty was met in achieving concordant results and in obtaining low " blank " values, and the precautions adopted are summarized briefly here. A simplified apparatus, similar to that described by Hancock and Laws (10) was used. The use of malonic acid for the production of carbon dioxide was considered to be unreliable, and the gas was led into the reaction flask from a cylinder using copper and polythene tubing. All solvents were distilled, with efficient fractionation, before use. The acetic acid used in the reduction was particularly troublesome and needed to be refluxed with chromium trioxide before distillation. It was found necessary to allow the reduction reaction to proceed for 2 hours, and vigorous shaking after the addition of the potash was required to ensure the full development of the violet colour. The colour was sensitive to light and the solutions were kept in the dark before measurement in the Unicam SP. 600 Spectrophotometer set at 625 mμ.

Method of Analysis.

As in previous work with DDT, in the examination of foliage differentiation has been made between the deposits residing on the surface and those that have passed within the leaf tissues. The leaves were washed with successive small portions of carbon tetrachloride to remove surface deposits, and were then air-dried, powdered and re-extracted in a percolator with hot solvent, to recover inside deposits. After the hot extraction, the apparatus was allowed to cool fully before removing the flask.

The solvent was then removed by passing a current of dry air over the solution which was kept at 25°C. To effect the oxidation of chlorbenside to the sulphone, the residue was dissolved with warming in 4 ml. of glacial acetic acid, 5 ml. of a 2·5 per cent. aqueous solution of potassium permanganate were added and the mixture was kept on a boiling water bath for 10 minutes. After cooling, freshly prepared 5 per cent. sodium metabisulphite solution was added, drop by drop, until the mixture was clear and colourless. Water was added, and the solution was extracted with three successive portions of carbon tetrachloride. Acetic acid retained by the solvent was neutralized by the addition of potash, drop by drop, with shaking. The carbon tetrachloride solution was then washed, dried with anhydrous sodium sulphate, concentrated to about 5 ml. with a current of air at 25°C. and passed through 5 g. of alumina as described in our method for DDT (5).

The percolate from the column was made up to volume, and aliquots taken for the determination of DDT and of *gamma*-BHC. For the *gamma*-BHC

estimation, a little liquid paraffin was added to prevent loss ; the solvent was then removed with a current of air at 25°C., and the residue was dissolved in glacial acetic acid for the reduction process. The optical densities of the colours in the DDT and *gamma*-BHC tests were measured spectrophotometrically, and the values obtained from calibration curves prepared from pure *pp'*-DDT and pure *gamma*-BHC.

To determine the chlorbenside, the alumina was freed from carbon tetrachloride by suction, and eluted with chloroform. Analysis of the chloroform solution was continued as in the DDT method. The nitrated compound was extracted with carbon tetrachloride, the solvent removed, the residue dissolved in a little benzene and the colour formed by the addition of 5 per cent. alcoholic potash. After measurement in the Hilger Spekker Photoelectric Absorptiometer (Ilford filter 604), the amount of chlorbenside was obtained from a standard curve prepared in tests using the pure sulphide.

The recovery of chlorbenside has been checked at all stages. Under the conditions of the tests, the extraction of both the nitrated DDT and nitrated sulphone with carbon tetrachloride is complete, but benzene may be preferred.

Application of the Method.

The appropriate steps of the method were used for different combinations of DDT, *gamma*-BHC and chlorbenside in spray deposits.

Soils rich in organic matter. In the analysis of most plant materials for DDT by our method, the use of alumina, and the rendering of the acid solution after nitration just alkaline to litmus, satisfactorily remove interference due to pigments, the blank determinations being of a very low order. A considerable number of soils and samples of grain have also been examined with no trouble.

A plant or grain extract, and more particularly a soil rich in organic matter, may, however, give a highly coloured carbon tetrachloride extract and some pigment may persist throughout the method to give an appreciable interference. When this occurs, we have found that permanganate oxidation of the residue after passage through the alumina is very beneficial, the blank determinations being reduced to low levels.

The possible movement of DDT into the edible portions of crops. Experiments with DDT on apple foliage reported earlier (5) showed that while surface deposits were largely lost over a period of 70 days, the inside deposits remained at approximately the same level. The DDT passing into the leaves soon after spraying may have persisted there ; on the other hand, there may have been a gradual movement of DDT into and from the leaves, accounting for the constant level of DDT within the tissues throughout the period. If this were so, the possibility arose of the movement of DDT from the leaves of plants such as peas and beans into the seeds and from the outer leaves into the heart of the cabbage.

To test this point, pea and horse-bean plants were sprayed to run-off with DDT emulsion at a dosage of 0·25 per cent. DDT. The foliage was analysed for outside and inside deposits of DDT over a period of 6 weeks. The initial surface deposits were of the order of 110–140 p.p.m., and fell to levels of 40 to 60 p.p.m. at the end of the period ; the inside deposits remained constant at 10–20 p.p.m. When the seeds from the pods were examined, no DDT could be found. The young outer leaves of cabbages were sprayed with DDT

emulsion at 0·25 per cent. DDT. When the developing hearts of the cabbages were analysed one month later, no DDT could be detected. There seems to be no risk of DDT accumulating in rapidly developing tissues such as those examined.

DDT, gamma-BHC and chlorbenside on apple foliage. Tests were made to ascertain the levels of the deposits and the behaviour on foliage of the components of the spring wash used by Kearns and Morgan. The DDT, *gamma*-BHC and chlorbenside, in an emulsion form, were each used at a dosage of 0·025 per cent., with 0·025 per cent. of succinate wetter. The wash was applied as a drenching spray.

The initial deposits of DDT, *gamma*-BHC and chlorbenside, determined soon after the spray film had dried, ranged from 89 to 96 p.p.m. of fresh leaf. Even after this short period, nearly 50 per cent. of the *gamma*-BHC deposited had passed within the leaf tissue, in contrast to the 5 per cent. of the DDT and the 10 per cent. of the chlorbenside that were found inside the leaves. Over a period of 14 days, the surface deposit of each compound decreased, with DDT showing the greatest persistence. The inside deposits of DDT and chlorbenside remained constant at a level of about 10 p.p.m., but the inside deposit of *gamma*-BHC quickly disappeared.

The results are of a preliminary nature and since they were obtained late in the season may not be representative of the behaviour of the spray chemicals on young foliage. Further work, including an examination of the extent of translocation of *gamma*-BHC, will be reported later

Summary.

Methods for the determination of spray deposits of DDT, *gamma*-BHC and chlorbenside have been examined, and a procedure for the determination of these chemicals occurring together on foliage is outlined. The use of the method in assessing the levels of deposits and the behaviour of the chemicals on sprayed crops is described.

REFERENCES.

(1) Kearns, H. G. H. and Morgan, N. G. (1954). *Ann. Rep. Long Ashton Res. Sta.*, 1953, 146–148.
(2) Bennett, S. H. (1954). *Ann. Rep. Long Ashton Res. Sta.*, 1953, 129–134.
(3) Kearns, H. G. H., Morgan, N. G. and Wilkins, A. H. (1955). *Ann. Rep. Long Ashton Res. Sta.*, 1954, 131–133.
(4) Cranham, J. E., Higgons, D. J. and Stevenson, H. A. (1953). *Chem. and Ind.*, 1206–1207.
(5) Martin, J. T. and Batt, R. F. (1954). *Ann. Rep. Long Ashton Res. Sta.*, 1953, 121–128.
(6) Schechter, M. S., Soloway, S. B., Hayes, R. A. and Haller, H. L. (1945). *Ind. Eng. Chem. Anal. Ed.*, **17,** 704–709.
(7) Schechter, M. S. and Hornstein, I. (1952). *Anal. Chem.*, **24,** 544–548.
(8) Higgons, D. J. and Kilbey, D. W. (1955). *J. Sci. Food Agric.*, **6,** 441–448.
(9) Higgons, D. J. and Kilbey, D. W. (1954). *Chem. and Ind.*, 1359–1360.
(10) Hancock, W. and Laws, E. Q. (1955). *The Analyst*, **80,** 665–674.

THE ROLE OF SUPPLEMENTS IN COPPER FUNGICIDE SPRAYS.

By E. SOMERS.

Supplements are often incorporated in fungicide sprays to improve their field performance. These supplements may be : protective colloids or deflocculating agents, used to delay the sedimentation of suspensions and to prevent their agglomeration ; wetters and spreaders, to facilitate contact between the spray and sprayed surface, thus improving the distribution of the deposit ; or stickers, to increase the adhesion of the fungicide deposit to the leaf or fruit surface. Some or all of these additives can be present in a commercial spray formulation and may have opposing effects on the ultimate field performance of the fungicide.

In recent work at Long Ashton a study has been made of the influence of some of these supplements on the spray retention of copper fungicides. Consideration has been given to the adhesion of weathered fungicide residues to surfaces, assessed in terms of each fungicide's " tenacity " (defined as the ratio of the amount of fungicide residue at a given time to its initial deposit), and to the effect of supplements on the level of deposits obtained after spraying to beyond " run-off."

Tenacity of Copper Fungicides.

The tenacity of a fungicide deposit on a plant surface is dependent upon the physico-chemical properties of the fungicide and surface, and upon the nature of the weathering to which the deposit is exposed. The literature on this subject, reviewed by Horsfall (1), is largely devoted to the behaviour of commercial formulations, which almost invariably contain surface-active agents, and it is well established (2) that these materials can greatly affect the tenacity of spray deposits.

Using pure samples of six copper compounds (cuprous oxide, cupric oxide, basic copper carbonate, copper oxychloride, Bordeaux and Burgundy mixtures) an investigation has been made (3, 4) of the influence of a number of physico-chemical factors on the tenacity of spray deposits on leaf and artificial surfaces. The technique for applying reproducible spray deposits, and for washing them with a jet of water for varying time intervals, has also been described (4, 5).

These investigations showed the influence of the following variables on the tenacity of a deposit :—

Type and size of deposit. When a surface is sprayed the discrete droplets that are formed agglomerate and eventually " run-off " leaving a partially or completely wetted surface. The tenacities of cupric and cuprous oxide deposits of equal initial amount, formed both above and below " run-off " levels of spraying, were found to be independent of the type of deposit. These results suggest that the discrete deposits produced by modern low-volume sprays will be no less tenacious than the " run-off " deposits given by the older high-volume sprays.

There is often an assumption in the literature that tenacity is independent of the magnitude of the deposit, but the present work has shown that tenacity is constant for a given surface and fungicide only at a defined surface concentration of the fungicide (expressed as weight of fungicide per unit area). Turner and Woodruff (6), and Rich (7) have suggested that the smaller the deposit the greater is the importance of the particle-surface adhesion but, with increasing thickness of deposit, particle-particle cohesion predominantly influences the tenacity. The present work showed that the tenacity on cellulose acetate of all the copper fungicides except Bordeaux initially decreased with increasing deposit then remained constant over the wide range 10-55 μg.Cu/sq.cm. (2-25 μg.Cu/sq.cm. for Burgundy), whilst for Bordeaux the tenacity was greatest at 18 μg.Cu/sq.cm. and decreased for both higher and lower initial deposits. From the above theory it would seem that the adhesion of the copper fungicide particles (except Bordeaux) to the surface is greater than their cohesion to each other.

Sprayed surface. Experiments on such different surfaces as cellulose acetate, paraffin wax (smooth and rough), and the leaves of bean, potato, tomato and cauliflower have shown that the tenacity of a deposit is not directly related to such physical properties of the surface as wettability, hairiness, or smoothness. The variation in tenacity on these surfaces seems to be more dependent on the chemical nature of the surface than on its microstructure.

Particle size of fungicide. As tenacity is a surface phenomenon, small particles should be more tenacious than large ones because of their greater surface area per unit weight and their greater deformability (8). Cupric oxide and copper oxychloride were found to behave in this manner on cellulose acetate, but for cuprous oxide and copper carbonate, on the same surface, decreasing the particle size was without effect on the tenacity, suggesting that the deformability of these particles was independent of size.

In vitro tests of the fungitoxicity, against *Alternaria tenuis*, of a number of cupric oxide size fractions showed that the biological effectiveness of the deposits (on cellulose acetate) increased with decreasing particle size until a diameter of 6 microns was reached, below which level there was no further increase of activity. It has been seen that, for cupric oxide, small particles are more tenacious than large, so that the particles of a polydisperse deposit will wash off at different rates. In practice this could mean, for materials behaving like cupric oxide on cellulose acetate, that the initial loss of deposit only slightly affects the biological activity of the residue, and emphasises that the exact relationship between the tenacity and fungitoxity of a fungicide is required to predict its field performance.

Type of weathering. The generally accepted view (1, 9) is that rain is the major agent of weathering : although wind is known to reduce dust deposits appreciably (10) its importance in removing spray deposits has not previously been assessed. The separate effects of wind and rain were resolved in a field experiment in which bean plants, sprayed with cuprous oxide and Burgundy, were protected from wind or rain by glass-sided boxes without sides or without tops, respectively. Although rain was found to be the major weathering agent the effect of wind was considerable. Wind was found to reduce the deposits by more than 50% after 27 days exposure, and its effect seemed more continuous than that of rain.

Effect of supplements on the tenacity of a copper fungicide.

Ever since the introduction of protectant sprays, stickers have been used in an attempt to improve the tenacity of spray deposits. The supplements used have been chosen on an *ad hoc* basis from such general adhesives as plant flours and gums, casein products, and natural resins. The ensuing literature is enormous; thus Harry (11) examined over 800 papers in his review of the subject. Unfortunately the majority of these papers are concerned with the testing of proprietary products of unspecified composition, and many others give no consideration to the effect of supplements on the biological efficiency of the fungicides. In the present work (4, 5), an examination has been made of the influence of a selected number of supplements of known composition on the tenacity and fungitoxicity of a copper fungicide, both in laboratory and field trials.

A screening test was devised whereby different types of supplements, recommended by previous workers, were incorporated with cupric oxide (chosen for its low tenacity compared with other copper fungicides) and sprayed onto a cellulose acetate surface. The spray deposit was then subjected to a standard washing procedure and its tenacity determined by analysis of the residue. Over 50 materials were tested in this manner but only a few gave an appreciable increase in the tenacity of cupric oxide. The most promising materials were the synthetic resins (coumarone resin, polyvinyl acetate and chloride (PVA and PVC)), the drying-type glyceride oils (linseed oil), agar, lime-casein, and rubber latex. Analysis of the residues showed that for these supplements the rates of " wash-off " for supplement and copper were of the same order, which suggests that the stickers had formed a homogeneous complex with the oxide rather than a coating over the deposit. The above materials were also found more effective than a number of proprietary stickers.

The seven stickers listed above were tested in 1953 with cupric oxide in a field trial on potato foliage. The tenacities of the spray deposits were determined chemically and the control afforded against blight (*Phytophthora infestans*) was used as a measure of their fungicidal efficiency. Little, if any, improved blight control was observed although most of the supplements increased the tenacity of the cupric oxide.

The extent to which the supplements interfered with the biological activity of cupric oxide was determined by *in vitro* assays against *Alternaria tenuis*; all the supplements except PVA and PVC were found to reduce the fungitoxicity of cupric oxide. This behaviour might be due to chemical interference with the reaction mechanism (thus the protein of lime casein competes with the fungus spores for copper (12)) or to such physical processes as the agglomeration of the fungicide into larger and therefore less active particles (see above) or to its " sealing-off " by the supplement. It is not always possible to distinguish between these two physical processes. Thus, sedimentation analysis showed that linseed oil flocculated cupric oxide and this could be as responsible for its inhibitory action as its formation of an impervious coating on the fungicide, suggested by Green and Goldsworthy (13) to account for poor fungus control when glyceride oils are used as supplements.

From this work only the latex-form synthetic resin emulsions, such as PVA, appeared promising stickers that were unlikely to reduce the potency of a cupric oxide fungicide. The low concentration (0·08% v/v) at which

PVA was used in the 1953 trials was considered to be responsible for its poor performance, for later laboratory experiments showed that it gave the most tenacious residues when used at a concentration of 1–2% v/v. Field experiments with these higher concentrations of PVA were carried out in 1955 (4) but the very dry summer prevented a critical test of the tenacities and fungicidal activities of the spray treatments. Further trials are needed in order to formulate a satisfactory spray incorporating a synthetic resin sticker.

Effect of supplements on the level of " run-off " deposits.

One of the most interesting results of the 1953 field experiments (5) was the way in which the magnitude of the cupric oxide deposit, formed after spraying to just beyond " run-off," varied with the type of supplement used : linseed oil, for instance, increased the initial deposit by a factor of 1·5 on potato, and 3·0 on tomato foliage. This effect has been ascribed by Marshall (14), and Fajans and Martin (15), to flocculation of the solid phase by oil emulsions and the subsequent preferential retention of the oil-wetted agglomerates by the sprayed surface. However, except for such emulsion systems there has been little consideration of the influence of supplements on the level of deposits formed after spraying to, and beyond " run-off." Evans and Martin (16) have shown that for solutions the maximum deposit is formed at the point of incipient " run-off." But, when high-volume sprays are applied mechanically in the field most of the foliage will be sprayed to considerably beyond " run-off."

A series of experiments has been carried out (4, 17) with representative wetting agents of anionic, non-ionic, and cationic type and the " run-off " deposits of cuprous oxide and Burgundy on leaf and artificial surfaces determined. When the surface was completely wetted by the spray suspension (zero receding contact angle) further spraying beyond " run-off " point did not alter the level of initial deposit. This level was found to increase when the viscosity of the spray fluid was raised by such supplements as glycerol and sodium alginate ; in such instances, when there is no preferential retention of the solid phase, the deposit remaining (on a vertically sprayed surface) will be largely determined by the viscosity of the spray and the rapidity of evaporation. However, substantially higher deposits were obtained only with solutions too viscous for practical application.

The different wetting agents gave deposit levels that varied with their type and concentration and also with the nature of the surface sprayed. For the succinate wetters the magnitude of the " run-off " deposit was found to decrease with increasing wetting ability of the spray (measured by the time of spraying required to produce " run-off "), and in general there was an optimum concentration range for each wetting agent on each surface. The highest deposits were given with polyethenoxy surface-active agents, perhaps because of their hygroscopic nature, the absorption of water retarding evaporation of the spray.

General conclusions.

The field performance of a protective fungicide spray is determined by the fungitoxicity, coverage, and the level of the spray deposit remaining after exposure to weathering. It has been seen, however, that a supplement that aids one of these factors may adversely affect the others. Thus, stickers may

increase the tenacity of a spray deposit but lower its fungicidal efficiency, whilst wetting agents may increase the coverage of a deposit but lower its initial deposit and also render it less resistant to removal by rain. Increased attention must be given to formulating sprays for particular purposes. Many sprays contain surface-active agents that disperse the fungicide in the tank but adversely affect spray retention, and a proper balance between these properties should be sought. This seems especially true of low-volume sprays, where complete wetting and " run-off " are not achieved. In this instance it may be advisable to use colloidal fungicides with these sprays or to incorporate a suitable dispersing agent that will dry to give an insoluble residue.

REFERENCES.

(1) Horsfall, J. G. (1945). *Fungicides and their Action* (Waltham Mass. : Chronica Botanica Co.).
(2) Fajans, E. and Martin, H. (1937). *J. Pomol.*, **15,** 1.
(3) Somers, E. and Thomas, W. D. E. *J. Sci. Food Agric.* (in the press).
(4) Somers, E. (1955). *Copper Fungicide Spray Deposits.* Ph.D. Thesis, University of Bristol.
(5) Somers, E. (1956). *J. Sci. Food Agric.*, **7,** 160.
(6) Turner, N. and Woodruff, N. (1948). *Bull. Connecticut agric. Exp. Sta.*, No. 524.
(7) Rich, S. (1954). *Phytopathology*, **44,** 203.
(8) Gregg, S. J. (1951). *The Surface Chemistry of Solids*, chap. III (London : Chapman and Hall).
(9) Martin, H. (1940). *The Scientific Principles of Plant Protection*, 3rd ed., (London : Arnold).
(10) Hopkins, L., Gyrisco, G. G. and Norton, L. B. (1952). *J. econ. Ent.*, **45,** 629.
(11) Harry, J. B. (1948). Ph.D. Thesis, Pennsylvania Univ. ; *Univ. Microfilms* (*Ann Arbor, Mich.*), Pub. No. 2139.
(12) Heuberger, J. W. and Horsfall, J. G. (1942). *Phytopathology*, **30,** 840.
(13) Green, E. L. and Goldsworthy, M. C. (1937). *Phytopathology*, **27,** 957.
(14) Marshall, J. (1937). *Bull. Washington agric. Exp. Sta.*, No. 350.
(15) Fajans, E. and Martin, H. (1938). *J. Pomol.*, **16,** 14.
(16) Evans, A. C. and Martin, H. (1935). *J. Pomol.*, **13,** 261.
(17) Somers, E. (in the press).

RADIOACTIVE TRACER STUDIES DURING THE PERIOD 1953-1955.

By C. P. LLOYD-JONES.

Introduction.

Research using radioactive isotopes has been in progress at Long Ashton since 1948. Initially the work was confined to radioactive phosphorus (^{32}P). which emits β-particles, detected and measured by the Geiger-Muller tube and scaler. Earlier papers (1 and 2) described the characteristics and the detection of β particles, explained the principles of radioactive tracer work, and gave accounts of investigations in crop nutrition and protection using ^{32}P.

More recently radioactive molybdenum (^{99}Mo), iron (^{59}Fe) and copper (^{64}Cu) have been used : these materials were obtained from the Atomic Energy Research Establishment at Harwell. Radioactive molybdenum emits β-particles and was measured by the Geiger-Muller counter. Radioactive iron emits high energy γ-rays and these γ-rays have been measured by the scintillation counter, which has recently become commercially available. Radioactive copper, iron and phosphorus have been detected in plant materials by the technique of autoradiography. It is proposed in this paper to outline the characteristics of γ-rays and to describe the scintillation counter and the autoradiographic technique. Examples of the application of the methods in plant studies are given and the advantages of the tracer technique over conventional methods are indicated.

γ-Rays.

γ-rays are electromagnetic radiations of short wavelength. Like X-rays they are characterized by their ability to penetrate matter, being gradually absorbed in the process. The absorption is greatest in dense material : most γ-rays fail to penetrate 20–30 cm. of lead. During the absorption electrons are released from the molecules of the material through which the rays are passing, and it is these electrons that the scintillation counter measures. Use may also be made of the freed electrons to measure the γ-rays by the Geiger-Muller counter, but this method is less efficient than the scintillation counter.

The Scintillation Counter.

In early work on radioactivity Rutherford used screens of zinc sulphide to detect nuclear particles. When a nuclear particle hit the screen a flash or scintillation appeared which was counted. The same principle is used to detect and measure γ-rays. The scintillation counter essentially comprises a phosphor, a photomultiplier and a scaler. The γ-rays produce flashes of light within the phosphor, the flashes are converted into electrical impulses by the photomultiplier and the scaler counts the impulses.

The scintillation counter is about 13 times more rapid than the Geiger-Muller tube (M6 type) when measuring small quantities, such as 10^{-2} μc ^{59}Fe in 10 ml. of solution, to the same precision. As the quantity of radioactive isotope is further reduced the effects of the higher efficiency become more marked, and for 10^{-4} μc ^{59}Fe in 10 ml. the scintillation method is about 30

times more rapid than the Geiger-Muller method. This increase in efficiency allows smaller quantities of such isotopes as ^{59}Fe, ^{60}Co and ^{65}Zn to be used, with a corresponding reduction in the risk of damage to plant tissues from radiation.

Autoradiography.

When a photographic film is subject to β or γ radiation (in the complete absence of ordinary light) " exposure " of the film occurs and on development in the usual way it will be found to be fogged. The autoradiographic technique involves applying a suitable photographic film to a leaf or a histological section.

After contact for some time in the dark, development of the film will show a dark pattern, revealing the distribution of the radioactive isotope in the leaf or section. The technique is not suitable for quantitative work but will give information on the location of radioactive isotopes that may not be obtainable otherwise.

The accuracy with which the autoradiographs can locate a source of activity in a tissue is referred to as the resolution, which is dependent upon (a) the distance between the film and the tissue, (b) the thickness of the tissue and (c) the thickness of the photographic emulsion.

The first factor is usually the most important. An autoradiograph of low resolution may be produced by placing a leaf on a photographic plate. A thin polythene sheet inserted between them will prevent the leaf from affecting the plate by scratching or chemical action.

Higher resolution is required to study the distribution within particular cells of a tissue. One technique involves mounting a section of plant tissue cut by microtome on a glass slide. A layer of fine-grain emulsion 5 μ thick, supported on gelatine 10 μ thick, is stripped in $1'' \times 1\frac{1}{2}''$ pieces from its photographic plate. One of these pieces is floated on water and the slide bearing the section is immersed in the water and raised when underneath the floating layer of emulsion. In this way the emulsion is brought into intimate contact with the section. The covered slide is dried, exposed in the dark, and developed, fixed and washed with the emulsion still firmly attached to the slide. Concentrations of silver grains may be seen in the emulsion over the radioactive cells when the section and emulsion are examined under a microscope. A phase contrast microscope is very convenient for the examination, the removal of the phase plate permitting the silver grains to be seen without interference from the section. The stripping emulsion technique has the disadvantage of not being suitable if the radioactive fraction is water-soluble.

Autoradiography has certain limitations. While the location of the isotope is shown, no information is given on its chemical form. For high resolution there must be a comparatively large amount of activity in the tissue, otherwise the lengthy exposure needed may be accompanied by fading of the latent image. The autoradiograph may be obscured by silver grains produced in the emulsion by chemicals from the tissue, or by pressure on, or scratching of the emulsion. The interpretation of results requires care, but with experience the method can yield valuable information.

Application and Advantages of Radioactive Tracer Methods.

In agricultural research the most important use of isotopes is as tracers : labelling a chemical by a radioactive isotope enables it to be traced or followed

during its passage into, or through a plant. There are at least five advantages possessed by the tracer technique over conventional chemical methods : (1) extremely small quantities of labelled material may be measured with accuracy ; (2) the radioactive label enables chemicals from different sources to be distinguished although they are for ordinary analytical purposes identical ; (3) while analysis by conventional chemical methods frequently involves chemical changes in the material, it is often possible to carry out radioassays that leave the chemical composition of the sample unchanged ; (4) the pattern of distribution of an isotope may be obtained on a film by autoradiography ; (5) in many investigations the use of labelled chemicals avoids lengthy chemical analysis and, by speeding up the measurements, enables more information to be obtained. Not all the advantages of the tracer technique will necessarily apply to a particular investigation, but examples to illustrate each of them can be drawn from work carried out at Long Ashton.

During experiments to investigate the behaviour of systemic insecticides it was frequently necessary to measure quantities of less than 1 μg of insecticide present in many grams of plant material. Using schradan and demeton-S labelled with radioactive phosphorus (^{32}P) the very small quantities present, for example in nectar, were measured with speed and accuracy (3, 4, 5).

The phosphorus taken in by a plant will come both from the soil and from phosphatic fertilizers. Once it is in the plant there is no chemical way of distinguishing between the phosphorus derived from the soil and from the fertilizer. The method of estimating the fertilizer uptake of plants by comparing the phosphate content of plants grown with and without fertilizer is open to the criticism that the extra phosphate content ascribed to fertilizer treatment may be due in part to soil phosphate uptake, the plant having been stimulated by the fertilizer to make more vigorous root growth. In an experiment to study the effect of induced iron chlorosis on fertilizer phosphate uptake the fertilizer was labelled with ^{32}P. The phosphate derived by the plant from the soil or the fertilizer was indistinguishable, but the radioactivity in the plant, coming only from the labelled fertilizer, permitted its uptake to be measured (6).

The non-destructive nature of the technique may be illustrated by reference to current investigations on the iron metabolism of micro-organisms grown in cultures containing iron labelled by ^{59}Fe. Iron was repeatedly extracted from the organisms and the iron remaining in the organisms after each extraction was determined by the tracer technique. A conventional iron analysis would have destroyed the sample.

Autoradiography has been used (7) to obtain information on the movement of a labelled systemic insecticide, demeton-S, when applied to leaves. Discrete spots of labelled demeton-S were placed on bean leaves : autoradiographs showed clearly the distribution of ^{32}P in the leaves and thus revealed the movement of demeton-S that had taken place in the three days after treatment (Fig. 1).

Finally, the use of tracers may result in a considerable saving of time. Frequently laborious analytical methods may be avoided, as in the work with systemic insecticides. Furthermore, this work would have required many more plants in order to provide sufficient insecticide residue for chemical analysis : the extra labour involved would inevitably have limited the information obtained.

PLATE IV

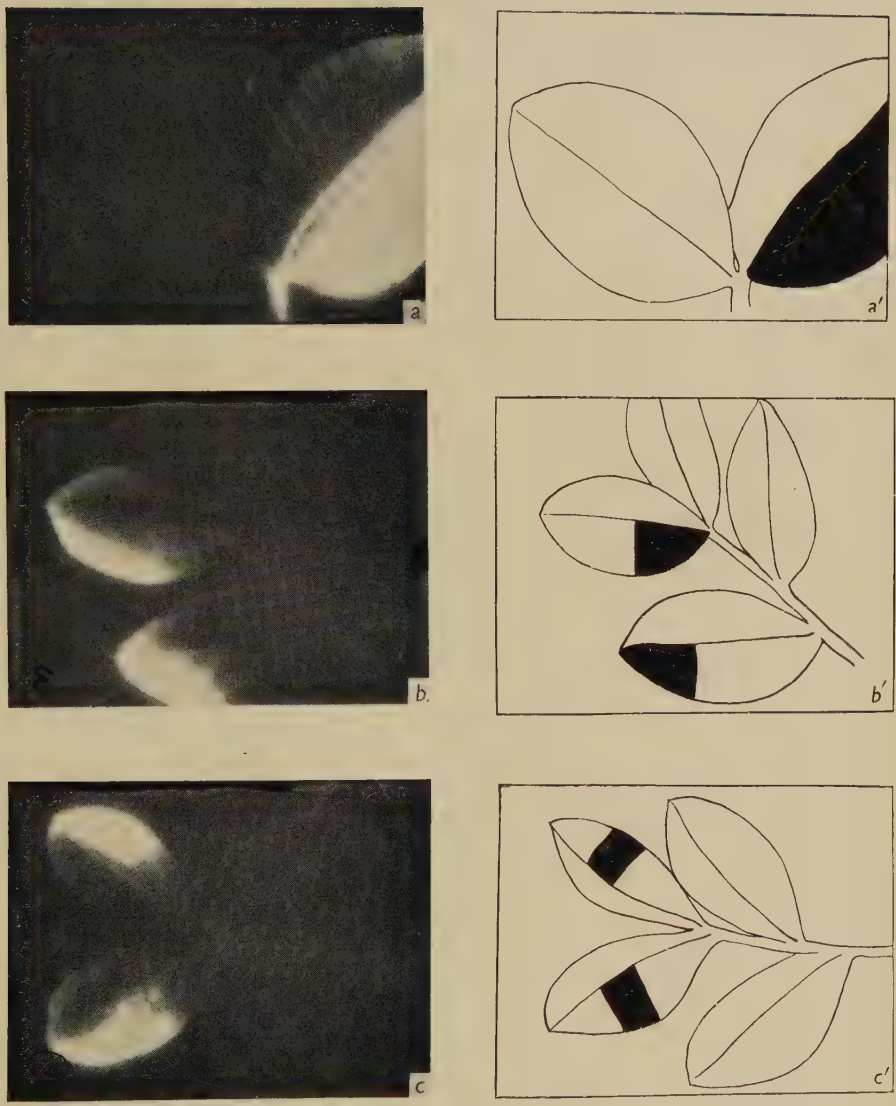


Fig. 1. Autoradiograph showing movement of demeton-S in bean leaves.
^{32}P-labelled demeton-S was applied to shaded areas, a′, b′ and c′.
a, b and c are prints from autoradiographs of the leaves harvested 3 days after application.
(Reproduced by courtesy of The Annals of Applied Biology.)

REFERENCES.

(1) Thomas, W. D. E. (1949). Radioactive isotopes an introduction to their properties and applications to plant nutritional studies. *Ann. Rep. Long Ashton Res. Sta.*, 1948, 114–123.

(2) —— (1954). Application of the radioactive tracer technique to problems in plant nutrition and crop protection. *Ann. Rep. Long Ashton Res. Sta.*, 1953, 102–107.

(3) Glynne Jones, G. D. and Thomas, W. D. E. (1953). Contamination of nectar with the systemic insecticide schradan. *Nature*, **171,** 263.

(4) —— and —— (1953). Experiments on the possible contamination of honey with schradan. *Ann. appl. Biol.*, **40,** 546.

(5) Thomas, W. D. E. and Glynne Jones, G. D. (1955). The systemic properties of diethyl-S-2-(ethylthioethyl) phosphorothiolate (demeton-S) with reference to the contamination of nectar. *Ann. appl. Biol.*, **43,** 182–191.

(6) Nicholas, D. J. D. and Thomas, W. D. E. (1953). Some effects of heavy metals on plants grown in soil culture. Part 7. The effect of cobalt on fertiliser and soil phosphate uptakes and the iron and cobalt status of tomato. *Plant and Soil*, **5,** 67–80.

(7) Thomas, W. D. E., Bennett, S. H. and Lloyd-Jones, C. P. (1955). The absorption, breakdown and systemic behaviour in plants of ^{32}P labelled demeton-S. *Ann. appl. Biol.*, **43,** 569–593.

AN UNUSUAL ATTACK ON APPLES BY A SAWFLY (*AMETASTEGIA* SP.).

By S. H. BENNETT.

In September 1955 sawfly larvae—*Ametastegia* sp.—were observed tunnelling into the pith of maiden and two-year-old apple trees of the varieties Cox's Orange Pippin and Lord Lambourne on M.II rootstocks and of Worcester Pearmain on M.VII. The orchard had been planted in arable during the winter of 1954–55 and grassed down in the spring of 1955.

The sites of larval entry were the main pruning cuts, and the larvae bored head first into the pith. The holes were bored to a depth of $\frac{1}{2}$–$\frac{3}{4}$ in. and in a few instances to 1 in. When the hole had been made sufficiently deep, the larvae turned round so that the head was outwards; the entrance hole was then sealed over with finely chewed tissue flush with the pruning cut. Below this outside seal was a short plug of coarser chips, and it was obvious that the site was chosen for hibernation. At the time of inspection, old holes were noted in some maiden shoots, suggesting that the larvae present in September were of at least the second generation.

The incidence of the pest was very high and in some rows borings had been made in over 70 per cent. of the leading shoots. Whilst in nearly all trees boring was confined to the maiden leader, on some of the two-year-old trees holes were bored into the original rootstock, into the first and second year leaders and some of the branch cuts. In a few trees borings into the side of the stem were observed, but these did not penetrate far into the wood.

It is not possible at present to assess the extent of the damage that may result from such an attack. Damage to the tissues by boring is not likely to be great though, as the hole usually penetrates below the point at which the leading shoot arises, there may be some weakening of the leader. The vertical holes in the stem become filled with water and, although the risk of establishment of cankers due to *Nectria* and *Gloeosporium*—which are essentially bark parasites—is not likely to be great, weakly parasitic or saprophytic fungi may well cause rotting of the waterlogged tissues, and death of the leading shoot may follow.

Theobald (1) reported similar damage from Ross in 1905, the species responsible being *Emphytus* (*Ametastegia*) *carpini* (Hart.), the larvae of which feed on *Geranium* spp. Larvae of the Dock Sawfly –*Ametastegia glabrata* Fall. —have often been recorded on apple fruits, into which they bore when seeking hibernation quarters, and Massee (2) has reported their tunnelling into the pithy wood of apple scions. The species involved in the present attack is not yet known: its identification awaits the emergence of adults in the spring. Larval feeding was not observed, but the commonest weed was Knotgrass—*Polygonum aviculare* Linn.—the leaves of which had been eaten.

Observations on the pest are continuing and it is hoped to publish a fuller account of its biology and of the damage it causes.

REFERENCES.

(1) Theobald, F. V. (1908). *Insect Pests of Fruit Trees*, London, p. 127.
(2) Massee, A. M. (1955). *Ann. Rep. East Malling Res. Sta.*, 1954, 134.

SPRAY APPLICATION PROBLEMS: XXIV. THE STUDY OF PLANT SURFACES AND SPRAY DEPOSITS BY MEANS OF TRANSPARENT IMPRESSIONS.

By S. H. BENNETT and C. G. L. FURMIDGE.

In the study of the performance of sprays it is important that the distribution of the active ingredient be studied on the actual plant surface sprayed. Methods are available for distribution studies on artificial surfaces such as glass or filter paper, and for convenience many workers have used these in place of plant surfaces. However, the distribution of spray material over such an artificial surface may differ considerably from its distribution over the actual plant. Martin (1) has described a plaster of Paris impression method for studying the distribution of lime-sulphur and copper deposits on plant surfaces. The main disadvantage in using these impressions, or the actual plant surface, lies in their opaqueness to transmitted light which restricts microscopic examination. The methods described in this paper, which are modifications of those given by Bryson (2) and Crisp and Thorpe (3), enable an accurate transparent impression of the plant surface to be obtained, which can then be readily enlarged by projection and examined visually or photographically. This enables a study to be made of the actual surface of the spray target, which varies considerably between plant species and even between varieties.

The method suggested by Bryson for the photomicrography of hard surfaces involved the use of a film of cellulose acetate softened with acetone to obtain an impression which, when hard, was projected and photographed. He suggested that this process was most applicable to specimens with a hard, rigid surface and that for softer and more delicate surfaces the method described by Crisp and Thorpe, who produced a similar type of impression by the use of a film of gelatin and glycerin, was more suitable. Both these methods have been examined with a view to their employment in the study of spray deposits and organisms on plant surfaces and, with certain modifications, both methods have been found to produce useful results. Some illustrations of impressions are given in this paper, but it should be emphasized that, due to loss of colour, their interpretation is not as easy as that of the originals from which they were taken.

Leaf Impressions.

1. *Cellulose acetate method.*

The best results have been obtained by using cellulose acetate film 0·005 in. thick ; a piece of this was attached to a glass plate of suitable size by moistening the underside of each end of the film with a 5·0% solution of cellulose acetate in acetone. A suitable area of the upper surface was then smeared with the cellulose acetate solution and almost immediately covered by the leaf, which was pressed very firmly in position. The pressure was continued for about 20 sec. and a further 30-60 sec. was allowed for the film to harden. After this

period the leaf was carefully peeled off, leaving an impression of its surface in the cellulose acetate film attached to the glass.

To obtain impressions of good definition the following factors have to be determined by practice :—

(a) The amount of acetone solution to smear on the film.
(b) The condition of the moistened film when the leaf is applied. If the surface is too wet, distortion of the film will occur, and if too dry, insufficient definition will be produced.
(c) The pressure required.

2. *Gelatin method.*

When using the gelatin-glycerin mixture suggested by Crisp and Thorpe, difficulty was experienced in separating the leaf from the gelatin film. It was found that a solution of approximately equal quantities by weight of gelatin and water gave satisfactory results. This mixture was heated in a waterbath at 60°C. until the mass was homogeneous. A small quantity was smeared on a slide previously warmed by placing it over a beaker of boiling water ; the leaf was then placed on the molten gelatin and lightly but thoroughly pressed into the gelatin film. When the gelatin had set hard (15–30 min. at room temperature) the leaf was carefully peeled off.

Owing to variations between different samples of gelatin, it is impossible to give exact proportions of gelatin to water, but a solution is required that rapidly sets hard on cooling. As with the cellulose acetate method, a certain amount of practice is required to obtain the best results.

Application of the Methods to the Study of Solid Spray Deposits.

Both methods produce a transparent negative of the surface contours of the leaf, recording considerable detail of the surface structure of the leaf together with any material attached to the leaf surface. Bryson (1) has shown that the accuracy of reproduction of surface detail is very high when the cellulose acetate method is used on a hard rigid surface. In the present work both methods have given excellent results, though the clarity and definition of cellulose acetate impressions have usually been superior to those on gelatin because of the greater pressure that may be employed with cellulose acetate. For the study of the characteristics of leaf surfaces very high pressures should be avoided since these may cause slight distortion of the surface detail, but any such distortion is not significant in the macroscopic study of spray deposits on plant surfaces.

It is possible to examine almost any solid spray deposit on plant material by using the appropriate method, the choice being governed by the nature of the deposit. Since acetone is used to moisten the surface of the cellulose acetate, this method cannot be employed to study deposits of any material that is soluble in this solvent. Such materials include most of the organic insecticides and fungicides and so for these the gelatin method has to be used. For similar reasons, the cellulose acetate method must be used for spray deposits that are water-soluble.

There is an important difference in the nature of the impressions obtained by the two methods. In the gelatin method all the deposit may not be removed, the remainder merely leaving an impression of its position in the gelatin,

whereas the cellulose acetate impression removes everything that is attached to the leaf surface, this material remaining embedded in the cellulose acetate. For this reason the latter method has proved ideal in studying deposits of the copper fungicides and of lime-sulphur. These materials are neither crystalline nor strongly coloured so they cannot be clearly distinguished in an impression. Since they are completely removed on the surface of the cellulose acetate, however, they may be developed by treating the impression with a solution that reacts with the deposit to produce a highly coloured compound.

Fig. 1 (a) shows the distribution of the deposit produced by a single drop (100 μ diam.) of Bordeaux mixture on the upper surface of a potato leaf. The copper has been developed with a solution of sodium diethyldithiocarbamate which converts the copper into a dark brown complex. The black areas are the particles of the copper complex radiating outwards from the point of impact and following the contours of the cuticle.

Lime sulphur may be developed with a solution of lead acetate which produces a black deposit as shown in Plate 1 (b). This is a low power photomicrograph of a cellulose acetate impression of the upper surface of a broad-bean leaf sprayed with discrete drops of 32% lime-sulphur, and the large black areas show the distribution of the spray deposit. The extreme sensitivity of the methods is well illustrated in Plate 1 (c) which is a gelatin impression showing the deposit obtained by spraying a broad-bean leaf with a suspension of BHC crystals in sulphite lye solution. Although the size of the individual crystals is only approximately 1·0 μ, their distribution on the leaf is clearly seen.

Although most of the experience with these methods has been gained by studying insecticides and fungicides on leaves, other plant surfaces can be studied. Satisfactory impressions can be made of strips of bark or fruit surfaces, as shown in Plates 1 (d) and 2 (a), which are cellulose acetate impressions of the bark of one-year-old wood of a flowering crab (*Pyrus mali*) and the skin of an apple fruit (Edward VII) respectively.

Further Applications of the Methods.

The use of these impression methods is not restricted to the study of spray deposits : they are also proving useful in examining organisms on the plant surface. For instance, it is possible to study the distribution and development of fungal spores by removal in a cellulose acetate impression and staining with a suitable dye. Plate 2 (b) shows ungerminated spores of *Botrytis fabae* stained with saffranin on a broad-bean leaf impression. After germination, lesions are formed and, as seen from Plate 2 (c), much of the surface structure of the leaf is destroyed by fungus attack. Since it is possible on a single impression to observe both the distribution of the fungicide and the spore development, the method is very useful in studying the range of action of fungicides.

The methods may also be used to study the density and distribution of the Red Spider mites ; a cellulose acetate impression removes from the leaf all stages of the mite, together with hatched eggs and cast skins, but these are easily distinguishable when examined by projection. Plate 2 (d) shows a cellulose acetate impression of various stages of *Tetranychus telarius* L. on a Tritonia leaf. Microscopic animals such as the free living stages of the Gall mites may also be studied.

The technique also promises to be useful in studying varietal characteristics of plant surfaces, either for purposes of identification or in relation to problems of spray impaction and retention.

Summary.

Simple methods of obtaining transparent impressions of plant surfaces in cellulose acetate and gelatin are described.

The detail of such impressions, which can be examined microscopically or by projection, renders them very valuable in the study of the nature of plant surfaces.

Some uses of the methods for the study of solid spray deposits, fungi and small animals on plants are described and illustrated.

Acknowledgments.

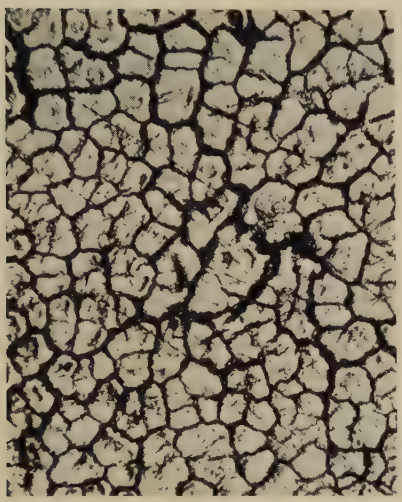
We are indebted to Dr. H. G. H. Kearns for his interest in this work and to Mr. N. G. Morgan who has employed the method in work on the biological evaluation of fungicide deposits and assisted in the preparation of Plates 2 (b) and 2 (c).

REFERENCES.

(1) Martin, J. T. (1953). *Ann. Rep. Long Ashton Res. Sta.*, 1952, 71.
(2) Bryson, H. Courtney (1954). *Lab. Practice*, Vol. 3, 377.
(3) Crisp, D. J. and Thorpe, W. H. (1950). *Nature*, **165**, 273.

PLATE VI.

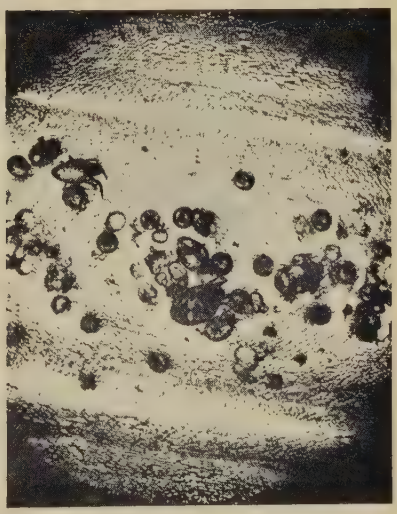
Fig. 2. TRANSPARENT IMPRESSIONS OF PLANT SURFACES (See p. 123).



(*a*) Skin of apple *var.* Edward VII. (×61)

(*b*) Spores of *Botrytis fabae* on broad-bean leaf. (×189)

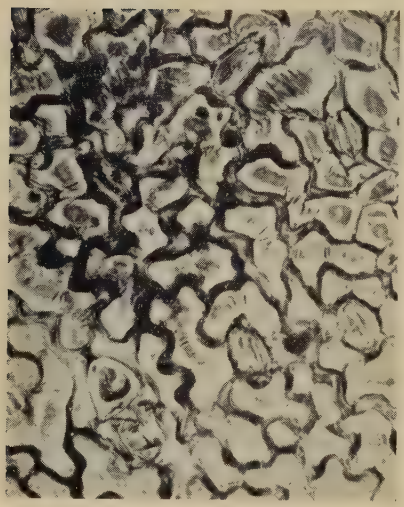
(*c*) Germinating spores of *Botrytis fabae* on broad-bean leaf. (×189)



(*d*) Various stages of mite *Tetranychus telarius* L. on leaf of *Tritonia* sp. (×15)

PLATE V.

Fig. 1. TRANSPARENT IMPRESSIONS OF PLANT SURFACES (See p. 123).



(*a*) Upper surface of potato leaf sprayed with Bordeaux mixture. (× 231)

(*b*) Upper surface of broad-bean leaf sprayed with 32% lime-sulphur. (× 15)

(*c*) Upper surface of broad-bean leaf sprayed with suspension of BHC crystals in sulphite lye. (× 225)

(*d*) Bark of one-year-old wood of crab-apple. (× 225)

SPRAY APPLICATION PROBLEMS: XXV. THE APPLICATION OF CHLORBENSIDE AND CHLORFENSONE FOR THE CONTROL OF THE WINTER EGGS OF THE RED SPIDER MITE (*METATETRANYCHUS ULMI*, KOCH).

By H. G. H. KEARNS, N. G. MORGAN and A. H. WILKINS.

A technique for assessing the ovicidal value of recently developed acaricidal materials against the winter eggs of the Red Spider mite has been described (1). It was found that 0·02 per cent. chlorbenside as a dispersible powder or benzol emulsion without a wetting agent gave a 99 per cent. kill of winter eggs provided it was applied just before egg hatch. The use of tubes closed at one end, to hold the pieces of one-year-old wood bearing the eggs, was open to the objection that the eggs were not subjected to the free circulation of air normally occurring in the field, and that this might have resulted in abnormally high kills.

Further experiments were carried out :—

(a) Comparing the hatch of treated eggs on wood placed in vertical tubes with no air circulation with that in horizontal tubes open at both ends and arranged with a free flow of air.

(b) Comparing chlorbenside (p-chlorbenzyl p-chlorphenyl sulphide) and chlorfensone (*p*-chlorphenyl *p*-chlorbenzene sulphonate).

Each of these materials was prepared as a benzol mayonnaise emulsion consisting of 8 oz. of the ovicide dissolved in 1½ pints of benzol emulsified with 1½ pints of 20 per cent. solution of sulphite lye of S.G. 1·3.

TABLE I.

THE CONTROL OF THE WINTER EGGS OF *Metatetranychus ulmi* BY CHLORBENSIDE AND CHLORFENSONE APPLIED 8/5/55.

Formulation	Tube closed one end, no air circulation	Open tube with full flow of air
	% hatch	% hatch
Chlorbenside emulsion alone—		
·05 per cent.	1·1	1·2
·02 per cent.	1·0	
Chlorbenside emulsion plus wetter—		
·05 per cent.	1·0	1·0
·02 per cent.		0·9
Chlorfensone emulsion alone—		
·02 per cent.	0·9	
Chlorfensone emulsion plus wetter—		
·05 per cent.		1·2
·02 per cent.	1·0	1·1
Control (untreated)	99·0	

Peak hatch of the eggs 13/5/55.

The ovicides were applied at 0·05 and 0·02 per cent., both with and without 0·025 per cent. dinonyl sodium sulphosuccinate wetter, at a time coinciding with the full pink bud stage of Worcester Pearmain apples. For each treatment a random sample of eight pieces of wood was taken, each bearing about 600–700 eggs. Treatments and results are shown in Table I.

The results confirm that the technique used for the breeding of the mite ensures a very high hatch of the untreated eggs, so that the percentage hatch of treated eggs gives an accurate measure of the effectiveness of the ovicides. By contrast, samples of eggs on wood removed from apple trees subjected to a natural Red Spider mite infestation show considerable lack of uniformity both in hatching and numbers. It is also evident that the placing of wood samples after treatment into vertical tubes with only the top end open does not cause any increase in control over a sample in freely circulating air.

A concentration of 0·2 per cent. of either chlorbenside or chlorfensone, prepared as benzol emulsions with or without a wetter and applied near egg hatch, gives a control of 99 per cent. of the winter eggs of the mite.

REFERENCE.

(1) Kearns, H. G. H., Morgan, N. G. and Wilkins, A. H. (1955). Spray application problems: XVIII. *Ann. Rep. Long Ashton Res. Sta.*, 1954, 131–133.

SPRAY APPLICATION PROBLEMS: XXVI. THE RANGE OF FUNGICIDAL ACTION OF MICRO-DEPOSITS OF CAPTAN ON ARTIFICIAL SURFACES AND ON LEAVES.

By D. J. BUTT.*

Previous articles in this series have stressed the importance of the form of distribution of protectant spray deposits, with special reference to copper fungicides. Morgan (1) used a micro-feed syringe to apply droplets of known size and concentration to bean leaves, which were later sown with a spore suspension of *Botrytis fabae* and incubated in humidity chambers. Provided that the spores remained linked by a water film to a copper micro-deposit, a zone of inhibition of germination was induced, its area depending on the distribution pattern of the fungicide.

In the present investigation, a similar method was used on pear leaves to explore the range of action of micro-deposits of captan against conidia of the pear scab fungus, *Venturia pirina*. Comparisons were also made of the effects of concentration and amount of the fungicide in the micro-deposits when applied to cellulose nitrate surfaces and to leaves. In the course of the work the possible movement of captan through leaf tissue was explored.

Materials and Methods.

In preliminary tests, captan (*N*-trichloromethylthiotetrahydrophthalimide) of 99% purity was employed but, as this could not be evenly maintained in suspension during use, most of the work was done with a 50% captan formulation containing a dispersing agent. Captan is commonly sprayed in the field at 0·1% (i.e. 0·2% of the 50% preparation) and this concentration, referred to below as "field strength," was the minimum used. Higher concentrations—twice, four times and eight times field strength—were also tested.

To provide a standardised artificial surface, glass slides were coated by dipping in 2·5% cellulose nitrate in butyl acetate, allowing to dry in a dust-free atmosphere and baking overnight at 80°C. For leaf tests, the translucent scale-leaves from the bases of unfolding pear buds were first used; later in the season young foliar leaves (var. Barnet) were chosen.

The appropriate captan suspension in water was applied to the coated slides or detached leaves with a micropipette delivering 0·0005 ml. drops of approximately 985 μ diameter. When the fungicide was used at field strength each drop on drying left a deposit of 0·5 μg. captan.

To obtain still smaller deposits, a "spinning disc" technique was used. The captan suspension was delivered at 1·5 ml. per second to the centre of a plastic disc, 10 in. diameter, revolving 2,500 times per second. The resulting droplets falling out in the zone 12 in. from the disc perimeter were of approximately 150 μ diameter, each depositing 0·002 μg. captan. A leaf or slide was

* Post-graduate research student, Horticultural Science Laboratory, University of Bristol.

The work reported in this paper was done at the Long Ashton Research Station in partial fulfilment of the requirements for the Bristol University Diploma in Plant Pathology and Protection.

placed to receive these droplets, the time of exposure being regulated by a shutter, and the area exposed being limited by a celluloid mask in which holes of 1·5 mm. diameter had been punched. By regulating the exposure time it was thus possible to allow a small number of discrete micro-deposits (countable under × 50 magnification) to impinge on a known area that could subsequently be covered by the spore inoculation drop.

The spores used for inoculation were conidia of *Venturia pirina* freshly obtained from young lesions on leaves of the variety Barnet. All spore suspensions were standardised at 50,000 per ml. tap-water, without added nutrient, and were used as 0·02 ml. drops unless otherwise stated. These inoculation drops were applied as required to the leaves or slides bearing the dried captan deposits and then incubated over water in closed Petri dishes.

After incubation the portions of leaf bearing the spores were mounted and carefully warmed in a lactophenol-cotton blue mixture which served both to clear the leaf tissues and to stain the germ-tubes without displacing the spores. Germination of the spores on the leaf surface could therefore be determined with reference to the position of any fungicide deposit present. Germination counts were made on 100 spores per drop and, in some experiments, germ-tube lengths were measured. Corresponding counts were made on the artificial surface.

Results.

Effects of Temperature and Substrate.

In preliminary experiments, made in the absence of a fungicide, it was shown that the optimum temperature for the germination of conidia of *V. pirina* was in the range 15° 20°C. Comparison on cellulose nitrate and on leaf surfaces showed a markedly quicker germination on the leaves (Table I), possibly due to the more favourable nutrient conditions on the leaf surface.

TABLE I.
COMPARISON OF SPORE GERMINATION ON CELLULOSE NITRATE AND ON LEAVES AT THREE TEMPERATURES.

Temperature °C.	Mean germ-tube length (μ)			
	after 18–20 hours		after 42–44 hours	
	on cellulose nitrate	on leaves	on cellulose nitrate	on leaves
10	5	23	28	45
15	32	69	—	—
20	26	41	53	84

Fungicide Effects.

Tests of movement through leaf tissues. Individual droplets depositing 0·5, 2·0 and 4·0 μg. captan were placed on the lower surfaces of both scale and foliage leaves and allowed to dry. Spore suspension drops were then placed on the upper surfaces so that the spores lay directly above the captan deposits,

being separated from them only by the thickness of the leaf. Controls were set up which differed only in having no captan deposit. The sown leaves were incubated at 20°C.

The results in Table II give no indication that captan on one surface of a leaf influences spore germination on the other surface.

TABLE II.
SPORE GERMINATIONS ON UPPER SURFACES OF LEAVES BEARING CAPTAN DEPOSIT ON UNDERSIDE.

Type of leaf	Time (hr.)	Captan deposit	Mean % spore germination
Foliar	18	none	53
„	„	0·5 μg.	70
„	„	2 μg.	67
„	„	4 μg.	53
Scale	46	none	47
„	„	0·5 μg.	42

In other experiments, drops of spore suspension on the upper leaf surface were arranged around a dried captan deposit (2 μg.) on the same surface. The distance between the spore drops and the captan was 1 mm., care being taken to avoid any water bridge between them. The results obtained, after 18 and 41 hours incubation, are shown in Table III.

TABLE III.
SPORE GERMINATIONS AT 1 MM. FROM CAPTAN (×4) DEPOSIT ON LEAVES : NO WATER BRIDGE PRESENT.

Time (hr.)	Germination %	Control, on leaves with no captan
18 ..	90	91
„ ..	84	87
„ ..	89	—
41 ..	94	94
„ ..	92	90

Again, these results give no support to the suggestion that sufficient captan to affect spore germination can travel through leaf tissue. This conclusion was supported by a chemical assay on the distal halves of pear leaves of which the proximal halves had been painted (on the tree) with a paste of 10% captan. After 24 hr. dry weather, twenty of the unpainted halves were taken, together with untreated controls, and assayed for captan by the resorcinol test (2).

No captan was found, indicating that no movement of the fungicide had taken place in the living leaves attached to the tree.

Range of action of captan deposits linked to spores by water bridge.

Experiments using deposits from micropipette. In these experiments the drops employed were of 0·0005 ml. of diluted captan at strengths from ×1 to ×8 (i.e. 0·1% to 0·8%). Each drop dried on leaves or coated slides to give an even circular deposit of approximately 1 mm. diameter.

Spore suspension drops were placed at distances from 1 to 6 mm. from the centre of the dried captan deposits and linked to the fungicide by a water bridge. After incubation at 20° for 18 or more hours, the percentages of spores germinating at various distances from the centre of the deposit were counted. The records are presented in Table IV and the mean results, after 18 hr., are represented graphically in Fig. 1.

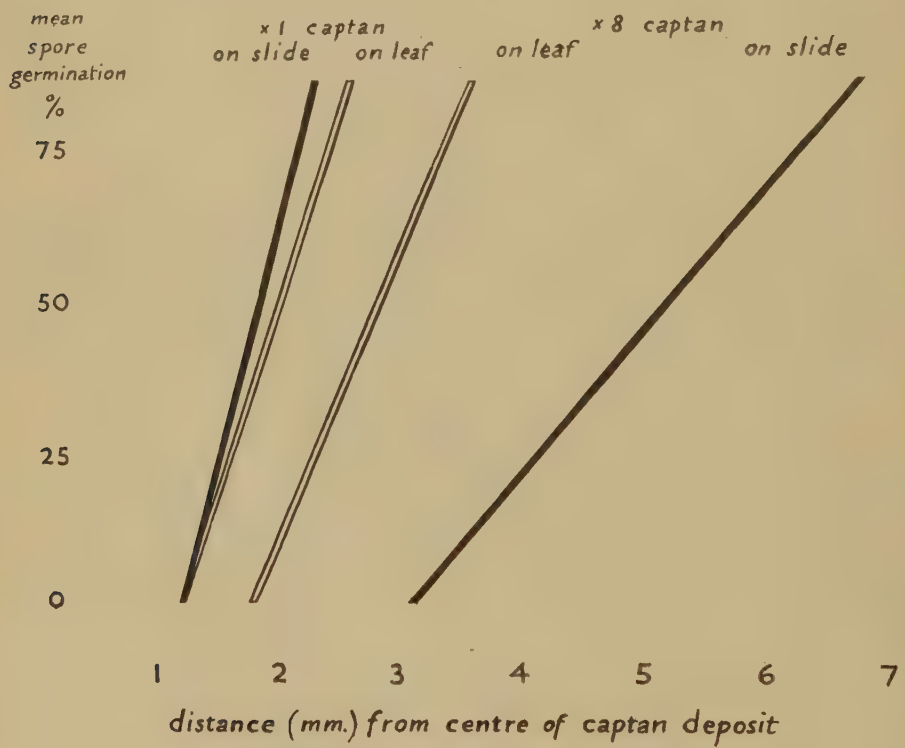


Fig. 1. Effects of surface and of fungicide concentration on range of action of water-linked captan deposits after 18 hour.

The results show a progressive increase in percentage germination with increasing distance from the captan deposit. With the ×1 captan after 18 hr., the zone of total inhibition has a radius of 1·2 mm. (area 4·55 sq. mm.) and the radius of 50% inhibition is approximately 2 mm. These values are almost identical on both slides and leaves. When the captan strength is increased 8 times, the radius of the zone of total inhibition rises to 3·1 mm. on slides

TABLE IV.

PERCENTAGE SPORE GERMINATION IN RELATION TO TIME, SURFACE, CONCENTRATION OF CAPTAN AND DISTANCE FROM CAPTAN DEPOSIT (LINKED TO SPORES BY WATER BRIDGE).

| Captan concn. | Time (hr.) | Surface | Distance (millimetres) from captan deposit | | |

(area 30·37 sq. mm.) and 1·8 mm. on leaves (area 10·24 sq. mm.). The corresponding radii for 50% inhibition are approximately 5·2 and 2·8 mm. respectively. Thus while the eightfold increase in captan concentration in the deposit multiplies the area of inhibition on the slide by approximately $7\frac{1}{2}$, the effect on the leaf is that this area is little more than doubled.

The effect of exposures longer than 18 hr. was examined, on slides only, in a limited number of experiments. As shown in Table IV, the inhibitory effect of the ×1 captan was almost entirely lost after 45 hr., suggesting that the 18-hr. effect was an arresting of germination rather than a killing of the spores. When the ×8 captan was used, the data obtained were insufficient to give the effect of time on the size of the area of total inhibition, but the figures suggest that the radius of the zone of 50% inhibition was reduced from approximately 5·2 mm. at 18 hr. to approximately 4 mm. at 45 hr. ; i.e. the area was roughly halved.

Experiments using deposits from spinning disc. The preceding section has dealt with fungicide drops of 0·0005 ml. (985 μ diameter) deposited by a micropipette and containing—at 0·1% concentration—approximately 0·5 μg. of captan. In contrast, the droplets generated by the spinning disc were of 150 μ diameter and contained, at the same concentration, only 0·002 μg. of captan. On a slide or a leaf these droplets leave a micro-deposit of approximately 240 μ diameter.

Table V gives the results obtained by placing spore inoculation drops (each 0·01 ml.) in contact with 1, 2 and 3 micro-deposits of ×4 captan on glass slides and leaves respectively.

When a spore suspension remains in contact with such a micro-deposit there is, apparently, no clearly-defined zone of non-germination bordering the captan particles. There is, however, a region of partial inhibition around the fungicide and, if more than one such micro-deposit is in contact with a spore inoculation drop of limited size there is evidence of a cumulative action. Thus, the depression of spore germination in an inoculation drop is dependent on the number of micro-deposits in the area covered by the drop.

TABLE V.

PERCENTAGE OF SPORES GERMINATING ON CELLULOSE NITRATE AND ON LEAVES IN PRESENCE OF MICRO-DEPOSITS (FROM 150 μ DIAM. DROPLETS) OF ×4 CAPTAN.

Surface	No. of micro-deposits	Percentage germination of spores after		
		17 hours	48 hours	96 hours
Slide	1	31	77	85
	2	4	9	13
	3	0	0	2
Leaf	1	86	92	
	2	63	—	
	3	—	82	
	5	31	—	

In Table V, as in Table IV, the contrast between the results on leaves and those on slides is apparent. Three $\times 4$ captan micro-deposits in contact with a spore suspension drop of 0·1 ml. were adequate to inhibit germination on a slide while, under comparable conditions on a leaf, five micro-deposits reduced germination only to 31%.

Further experiments were made on leaves, using from one to ten micro-deposits of captan at $\times 8$ strength, allowed to impinge on circular areas of 1·5 mm. diameter delimited by the holes punched in a celluloid mask. As the diameter of each dried micro-deposit was approximately 240 μ, the percentage areas covered by the micro-deposits could be estimated. These values are included with the spore germination results given in Table VI.

TABLE VI.

PERCENTAGE OF SPORES GERMINATING ON LEAVES IN PRESENCE OF MICRO-DEPOSITS (FROM 150 μ DIAM. DROPLETS) OF $\times 8$ CAPTAN.

No. of micro-deposits per 1·76 mm².	Percentage area covered	Percentage germination of spores after			
		17 hours	22 hours	41 hours	44 hours
1	2	—	—	—	86
2	5	33	85	86	81
3	7	36	—	91	—
4	10	—	12	77	64
5	12	8	12	—	22
6	15	2	12	—	55
7	17	—	11	—	—
8	19	—	—	—	31
9	22	—	—	—	31
10	24	—	—	—	16

(Dashes signify no test).

Table VI illustrates the falling-off of the fungicidal performance of the micro-deposits with lapse of time. Up to 17 hr. a distribution of micro-deposits covering approximately 10% of the leaf surface inhibits the germination of nine-tenths of the viable spores, but after 44 hr. a cover of 24% inhibits only five-sixths.

A comparison of the effects of the absolute amounts of captan available to the spores, irrespective of the concentrations used in applying the microt deposits, can be made in terms of parts per million of captan with which the spore inoculation drop is in contact. A 0·01 ml. drop covering one micro-deposi-from a $\times 1$ captan droplet is in contact with 0·002 μg. captan. Captan is therefore available in the spore drop at 0·2 p.p.m. If the captan is deposited at $\times 4$ strength, the corresponding figure will be 0·8 p.p.m. and for $\times 8$ captan, 1·6 p.p.m. Each additional micro-deposit raises the total by an equal increment.

This relationship between number of micro-deposits, strengths of captan applied, and p.p.m. captan in contact with the spore inoculation drop is illustrated in Fig. 2, which assembles the comparable germination records from Tables V and VI.

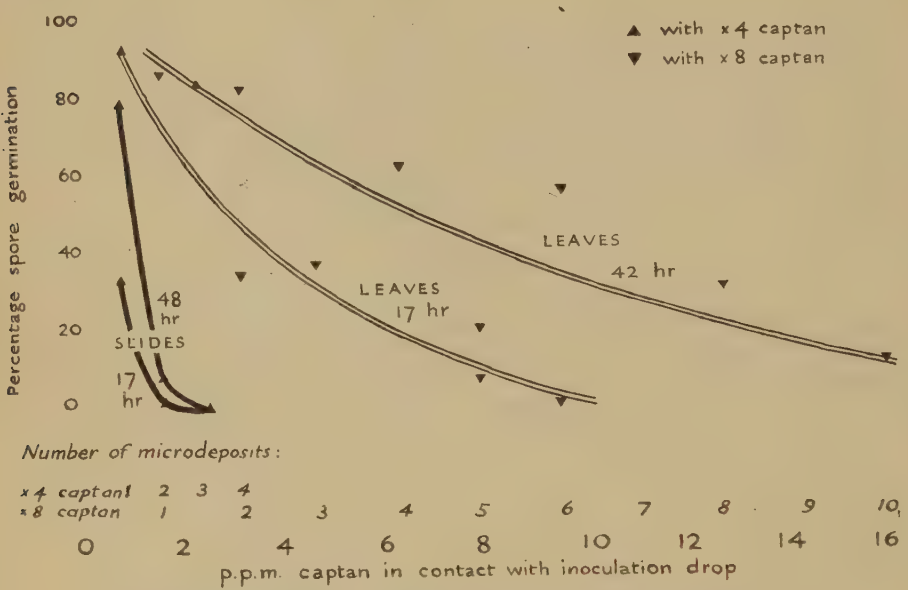


Fig. 2. Spore germination records from Tables V and VI plotted against p.p.m. captan in micro-deposits.

Fig. 2 again emphasizes the contrast between the results obtained on slides and on leaves. For example, after 17 hr., complete inhibition of spore germination on slides is shown at 2·4 p.p.m., while on leaves approximately 11·2 p.p.m. is required to secure complete inhibition after the same period.

The level for 50% inhibition of spore germination on slides is shown by Fig. 2 to be approximately 1·1 p.p.m. This is of the same order of magnitude as the figure (0·89 p.p.m.) given by Byrde (3) as the ED 50 level for captan used in a slide test against spores of *Sclerotinia laxa*.

Discussion.

As no evidence of the movement of captan in leaf tissue was found in these trials, it is assumed that its fungicidal action is exerted through the water layer linking the captan deposit to the spores. Photomicrographs of captan deposits in water, taken at 24-hr. intervals, suggest that particles of the fungicide may become detached and redeposited near the periphery of the original residue. Such redistribution indicates that the adherence of particles of captan one to another may be less than the adherence between captan and the surface on which it is deposited. This property would clearly be strongly affected by the presence of any surface-active material, and it should be stressed that the results reported in this paper were obtained with a captan formulation that included a dispersing agent. In the absence of this agent, the zones of inhibitory effect shown by captan deposits would possibly be less marked.

The existence of zones of fungicidal action around discrete micro-deposits is of practical interest in relation to field application of small-volume sprays from mist-blowers. The optimum droplet diameter in spray mists for fruit-trees is probably of the order of 60–80 μ, but many of the dispersions in actual use are made up of droplets above this size. In the spinning-disc experiments recorded in Tables V and VI micro-deposits from droplets of 150 μ diameter gave satisfactory inhibition of spore germination on leaves for a period of 17 hr., provided that the area covered by the fungicide was of the order of 15% of the whole. It is therefore probable that such a distribution of micro-deposits would give satisfactory scab control in the field. However, reference in Table VI to the unsatisfactory results obtained from a cover of less than 10% suggests that the 15% figure is approaching the lowest level of effective performance and so allows little margin for defects in evenness of distribution of micro-deposits of this size.

Acknowledgments.

Grateful acknowledgment is made to Mr. N. G. Morgan for helpful suggestions and for the loan of the spinning disc assembly, to Mr. J. A. Pickard for chemical assays of captan on leaves, and to Mr. R. W. Marsh, under whose direction this work was carried out.

REFERENCES.

(1) Morgan, N. G. (1953). Spray application problems : VI. A laboratory technique using *Botrytis fabae* on broad bean for the biological evaluation of fungicidal spray deposits. *Ann. Rep. Long Ashton Res. Sta.*, 1952, 99.

(2) Martin, J. T. and Pickard, J. A. (1955). Spray application problems : XIII. Determination of captan deposits : progress report. *Ann. Rep. Long Ashton Res. Sta.*, 1954, 83.

(3) Byrde, R. J. W. (1952). Experiments on the control of Brown rot of apples and plums : I. Laboratory tests. *J. hort. Sci.*, **27**, 130.

SPRAY APPLICATION PROBLEMS: XXVII. FIELD EXPERIMENTS IN THE CONTROL OF APPLE AND PEAR SCAB BY SMALL-VOLUME SPRAYS, 1955.

By H. G. H. KEARNS, R. W. MARSH and N. G. MORGAN.

The 1954 trials in this series (1) showed that, on large bush trees of Cox and Worcester, scab control was unsatisfactory after five small-volume air-flow applications at 50 gal. per acre, using 1% or 2% lime sulphur or 0·1% captan. Large-volume spraying (500 gal. per acre) with 1% lime sulphur gave virtually complete scab control but caused serious russeting of Cox.

In preliminary trials on pears in 1954 (not previously reported) both small-volume and large-volume treatments gave a high measure of scab control, but the large-volume 1% lime sulphur caused extensive leaf scorching in June.

In 1955 the trials on pears were continued, 1% lime sulphur being compared at 30, 80 and 250 gal. per acre. Applications of captan (0·1% and 0·4%) at 30 gal. per acre were also included. Di-octyl sodium sulphosuccinate wetting agent (0·025%) was used in all the treatments.

On the apple plots no large-volume sprays were used in 1955. Six small-volume applications (55 gal. per acre) were made, comparing 2% lime sulphur, 4% lime sulphur and 0·1% captan.

Experimental.

The apple trials were made on large 30-year-old bush trees of Cox and Worcester on Plots 14 and 15. A description of these plots was given in an earlier paper (2).

The pear plot (Plot 9), of 4 acres, was planted 1934–5 and grassed down in 1947. The trees form thirty-two rows 15 ft. apart with a planting distance of 12 ft. in the rows. The principal varieties are Conference, William's, Laxton's Superb, Beurré Bedford, Doyenné du Comice, Beurré Hardy, Bristol Cross, Durondeau and Dr. Jules Guyot. Seven rootstocks are represented in the plot, which has been fully described by Hobbis and Catlow (3, 4). The close planting of the trees in the rows, and the policy followed of restricting branch growth across the alleys, greatly facilitated spraying operations in this plot.

The sprays were applied as described in the previous report, except that on the pears the small-volume sprays were applied at 30 gal. per acre and the large-volume at 250 gal. per acre. A " medium-volume " air-flow application (80 gal. per acre) was also included on the pear plot. The lime sulphur employed was a standard proprietary product and captan was used as a 50% proprietary formulation : the use of this material at 8 lb. per 100 gal. caused difficulties owing to rapid sedimentation. The complete spraying programmes are set out in Tables I and II.

Up to mid-June, the 1955 season was favourable to scab, but the rest of the summer was exceptionally dry and sunny. Rainfall in inches (with the 1914–53 means for comparison) was as follows : May 3·91 (2·59), June 2·59 (2·11), July 0·15 (3·16), August 0·64 (3·65), September 1·34 (3·15).

Results.

On the apples, fruit counts and percentage scabbed area per fruit were recorded on 23 September (Worcesters) and 30 September (Cox). (See Table I). In the pear plot, the percentage scabbed area per fruit was recorded for all the trees of William's on 25 September (See Table II).

The percentage weights of scabbed fruits harvested from both apple and pear trial plots are also shown in Tables I and II.

Discussion.

The outstanding feature of these trials is the difference between the results of the small-volume trials on the two plots. On the apples, the level of scab control achieved by the small-volume sprays was unsatisfactory, even when lime sulphur was used at four times the standard strength. On the pears, on the other hand, the control of scab by the small-volume captan sprays was almost perfect, when the captan was used both at standard and at quadruple strength. Lime sulphur at 1% applied to the pears at 30 gal. per acre gave poorer scab control than the same material at 80 or 250 gal. per acre but still showed a wide measure of advantage over the " no spray " treatment.

Of the possible causes for this contrast between the results on the apples and those on the pears, differences in skill of application can be ruled out, since all the small-volume spraying was done by the same team and equipment. The timing of the applications on the apple plot is open to criticism in that it shows a gap of 20 days between 11 and 31 May, whereas there is no gap longer than 15 days in the pear spraying programme. The major difference between the two plots lies, however, in the shape and spacing of the trees. The diameter of the heads of the apple trees is approximately 20 feet, and the proximity of the branches of adjacent trees hinders air-flow application. In the pear plot, the tree heads, viewed from above, are oval : the diameter of the head, taken at right angles to the rows, is approximately 5 feet, while the unimpeded alleys and hedge-like conformation of the lines of trees favour air-flow application. Thus, in the pears the distance of travel of the spray droplets into the head of the tree was required to be only one-quarter of the corresponding figure for the apples. This factor, depending on tree layout and shaping, is thought to provide the major reason for the better performance of the small-volume sprays on the pears.

Summary.

Small-volume air-flow sprays of lime sulphur and captan, at 30–55 gal. per acre, were used in field trials against apple and pear scab at Long Ashton in 1955.

Unsatisfactory scab control was achieved by six applications of either 2% or 4% lime sulphur or 0·1% captan, at 55 gal. per acre per application, to 30-year-old bush trees of Cox and Worcester, having heads approximately 20 ft. in diameter.

Excellent control of pear scab was obtained by six applications of 0·1% captan, at 30 gal. per acre, to William's pears grown in a modified " hedgerow " system in which the transverse diameter of the tree heads was approximately 5 ft. The better performance of the small-volume sprays on the pears is ascribed to the more suitable conformation of the trees for this type of application.

TABLE I.

RELATION OF FUNGICIDE CONCENTRATION, METHODS, TIME AND NUMBER OF APPLICATIONS OF LIME SULPHUR AND CAPTAN TO SPRAY DAMAGE AND APPLE SCAB CONTROL.

Method of application	Approx. gal. per acre	Fungicide per 100 gal. wash		*Application dates	Mean % scabbed area on fruit		% wt. scabbed fruit at harvest		Mean No. fruits per tree Cox
					Worc.	Cox	Worc.	Cox	
Small-volume air-flow	55	Lime-sulphur	2 gal.	1, 2, 3, 4, 5, 6	0·52	0·25	22·0	32·6	89·5
Small-volume air-flow	55	Lime-sulphur	4 gal.		0·31	0·16	28·0	56·0	86·5
Small-volume air-flow	55	Captan	2 lb.		0·59	0·27	47·0	66·0	80·0
Unsprayed	—	—	—	—	0·80	0·37	60·0	61·5	44·3

TABLE II.

RELATION OF FUNGICIDE CONCENTRATION, METHODS, TIME AND NUMBER OF APPLICATIONS OF LIME SULPHUR AND CAPTAN TO SPRAY DAMAGE AND PEAR SCAB CONTROL.

Method of application	Approx. gal. per acre	Fungicide per 100 gal. wash		Application dates*	Mean % scabbed area on fruit of William's	% wt. scabbed fruit at harvest	Mean No. fruits per tree
Large-volume automatic ..	250	Lime sulphur	1 gal.	7, 8, 9, 10, 11, 12	0·12	12·0	50·6
Medium-volume air-flow ..	80	Lime sulphur	1 gal.		0·10	12·3	29·8
Small-volume air-flow	30	Lime sulphur	1 gal.		0·29	32·2	52·2
Small-volume air-flow	30	Captan	2 lb.		0·04	2·0	57·0
Small-volume air-flow	30	Captan	8 lb.		0·05	0·0	64·8
Large-volume hand-lance ..	250	Captan	2 lb.		0·01	0·0	46·4
Unsprayed	—	—	—	—	0·97	71·3	34·7

* Key to application dates : (1) May 11 (2) May 31 (3) June 10 (4) June 17 (5) June 30 (6) July 7 (7) April 27 (8) May 12 (9) May 25 (10) June 9 (11) June 22 (12) June 30

Acknowledgments.

Thanks are due to Messrs. C. W. Harper and J. H. Russell for assistance with spraying and fruit counts and to Mr. R. T. Burchill for help in recording.

REFERENCES.

(1) Kearns, H. G. H., Marsh, R. W. and Morgan, N. G. (1955). Spray application problems : XIX. Further field experiments on the control of apple scab by small-volume sprays. *Ann. Rep. Long Ashton Res. Sta.*, 1954, 134–137.

(2) Kearns, H. G. H., Marsh, R. W. and Morgan, N. G. (1953). Spray application problems : IV. Preliminary field experiments on the control of apple scab by small-volume sprays. *Ann. Rep. Long Ashton Res. Sta.*, 1952, 90–95.

(3) Hobbis, E. W. and Catlow, E. (1945). The performance of certain varieties of pear on various rootstocks at Long Ashton : progress report I. *Ann. Rep. Long Ashton Res. Sta.*, 1944, 21–30.

(4) Hobbis, E. W. and Catlow, E. (1950). Tree shape and pruning treatment in relation to crop yield in pears : progress report. *Ann. Rep. Long Ashton Res. Sta.*, 1949, 34–39.

SPRAYING EXPERIMENTS AGAINST APPLE SCAB AT LONG ASHTON, 1955.

By R. J. W. BYRDE, G. M. CLARKE and C. W. HARPER.

This report describes the second year of a three-year trial designed to assess the relative performance of captan and lime sulphur as post-blossom fungicides on the apple variety Cox's Orange Pippin. In the first year of the trial (1) both materials afforded an excellent control of apple scab (caused by *Venturia inaequalis* (Cooke) Wint.) in a wet season. There was a significantly higher incidence of apple mildew (caused by *Podosphaera leucotricha* (Ell. and Everh.) Salm.) on the shoots of the 1954 season's growth on the plots receiving captan ; on the other hand, the substitution of captan for lime sulphur led to a significant reduction in fruit russet (which was severe on the lime sulphur plots) and to an improvement in fruit size.

Several reports of the performance of captan during 1955 have already appeared in the technical press. In general these corroborate previous findings that captan controls scab satisfactorily but has no effect on mildew or Cox spot. The value of captan in a dry season under conditions in the West of England is discussed below in the light of data obtained at Long Ashton in 1955.

Experimental.

Fungicides. As in 1954, the spray materials were formulated as follows :—

Lime sulphur, as the commercial concentrate diluted to 2% or 1% (pre-blossom) and to ¾% (post-blossom).

Captan (N-trichloromethylthio-tetrahydrophthalimide), as an aqueous suspension of a commercial wettable powder (50% captan) used at 1½ lb. per 100 gal.

The sprays were applied at approx. 260 gal./acre by a mobile spraying machine using two twin-nozzle hand-directed brooms with 4/64 in. disc apertures, and short-range swirl plates. The output of each broom was approx. 3½ gal./min. Details of the spray applications during 1955 are given in Table I.

Layout. The general layout of the site was described in the preceding paper (1). In 1955, treatments were re-allocated to the plots, as indicated in Table II. Each experimental plot consists of the full length of four rows, extending over three planting systems, which are recorded separately. These planting systems are referred to as IIA (type II rootstock without " fillers "), IIB (type II rootstock with " fillers ") and IXB (type IX rootstock, used as " fillers "). All records were taken on the variety Cox's Orange Pippin.

Methods of assessment.

Apple scab was assessed on fruits by Tehon and Stout's method, as amplified by Marsh (2). No assessment of leaf scab was made.

Apple mildew. (a) An estimate of mildew infection on blossom trusses in late spring was made by a sampling method. Six trees, chosen at random, were recorded in each experimental plot in each planting system, five trusses being examined per tree and classed as " infected " (if any mildew observed) or " healthy." The position of the five trusses, taken from a single branch,

TABLE I.

SPRAY APPLICATIONS DURING 1955 : QUANTITIES PER 100 GAL. WASH.

Date	Stage	Fungicide	Insecticide or Acaricide	Wetting Agent
April 26	Green cluster	Lime sulphur 2 gal.	DDT (a) 4 oz. *gamma* BHC (a) 1⅛ oz.	Dioctyl sodium sulphosuccinate (b) 4 oz.
May 12	Pink bud	Lime sulphur 1 gal.	—	—
June 1	Petal fall	(i) Lime sulphur ¾ gal. *or* (ii) Captan (50%) 1½ lb.	*gamma* BHC (a) 2¼ oz.	—
June 17	Fruitlet	As above	—	—
July 5	"Codlin"	As above	DDT (a) 8 oz. Chlorbenside (a) 3¼ oz.	Dioctyl sodium sulphosuccinate (b) 1 oz.
Aug. 30	(c)	—	Chlorbenside (a) 3¼ oz. Rotenone (25%) (b) 3¼ oz.	Dioctyl sodium sulphosuccinate (b) 1½ oz.

(a) As benzene emulsion. (b) In acetone solution.
(c) To deal with outbreak of red spider following prolonged dry spell. Pre-harvest spray was applied over the whole plot on Sept. 7, using α-naphthalene acetic acid (10 p.p.m.) and wetting agent.

was determined by assigning two random numbers to each selected tree. The first of these was used to give the orientation of the desired branch, and the second to give both height of branch and whether on inner or outer part of the tree.

(b) Mildew infection of the current season's growth was estimated in late summer by an examination of ten shoots per tree. Each was recorded as "infected" or "healthy."

Crop weight was recorded for each tree to the nearest 0·1 lb.

Fruit russet, as percentage area of the total fruit surface, was estimated by visual comparison with models showing 10% and 25% area affected, in three categories : (A) less than 10%, (B) 10% to 25%, (C) more than 25%.

Fruit grading for size and colour was carried out on a commercial machine.

Notes on weather.

The summer of 1955 was exceptionally dry after mid-June. The rainfall figures (in.) for each month are shown below, with average figures (1914–53) in brackets :—

April, 1·42 (2·33) ; May, 3·91 (2·59) ; June, 2·59 (2·11) ; July, 0·15 (3·16) ; August, 0·64 (3·65) ; September, 1·35 (3·15).

Total, 10.06 (16.99). Deficit, 40.8%.

After a period in late May and early June favourable to scab development, prolonged dry weather checked further spread of the disease. On unsprayed trees of the variety Cox's Orange Pippin elsewhere in the Long Ashton plantations, the level of fruit scab infection was only moderate.

Results.

On foliage and blossom trusses.

Apple mildew was assessed on blossom trusses on 1 June, 1955. 90 trusses were examined per treatment in each planting system. The numbers of infected trusses for systems IIA, IIB and IXB respectively were 0, 0, 0 for lime sulphur, and 0, 1, 1 for captan. These results, obtained before the application of differential spray treatments in 1955, reflect only the 1954 treatments.

A further assessment of apple mildew was carried out on shoots of the current season's growth on 14–15 September, with the results shown in Table II. These show no indication either of different effects of the 1955 treatments or of residual effects of the 1954 treatments, except that on the plots which received lime sulphur at all stages in both seasons incidence is strikingly low.

TABLE II.

PERCENTAGE SHOOTS OF CURRENT SEASON'S GROWTH SHOWING MILDEW INFECTION, SEPTEMBER 14–15, 1955.

Block	Post-blossom fungicide*		Planting system		
	1954	1955	IIA	IIB	IXB
1	LS	LS	10	12	8
	C	C	35	48	53
2	LS	C	47	35	40
	C	LS	33	20	53
3	C	LS	43	37	40
	LS	C	53	37	42
Overall means for 1955 treatments		LS	29	23	34
		C	45	40	45

* LS = Lime sulphur; C = Captan.
Plots are listed down the table from north to south.

On fruit.

The fruit of the variety Cox's Orange Pippin was picked on September 27–29. The total fruit from each recorded plot was bulked in three batches corresponding to planting systems IIA, IIB and IXB, and random samples of about sixty fruits were taken from orchard boxes by the method of Pearce (3).

TABLE III.

PERCENTAGE FRUIT AREA SCABBED ON SAMPLES, OCTOBER 3, 1955.

1955 post-blossom fungicide	Planting system		
	IIA	IIB	IXB
Lime sulphur	0·04	0·02	0·01
Captan	0·02	0·00	0·00

Scab was assessed on these samples on October 3; the results are summarized in Table III. Owing to the very low incidence of the disease, no statistical analysis was carried out.

Fruit russet was also estimated on these samples; the results are summarized in Table IV. Inspection of the full data showed no indication that the 1954 treatments had had a residual effect.

TABLE IV.

DISTRIBUTION OF FRUIT RUSSET ON SAMPLES, OCTOBER 3, 1955.

Planting system	1955 post-blossom fungicide	No. fruit russeted in each category			Category (A) as percentage of total	
		(A) <10%	(B) 10% to 25%	(C) >25%		
IIA ..	Lime sulphur	152	2	—	99	
	Captan	153	1	—	99	
IIB ..	Lime sulphur	182	8	—	96	*
	Captan	190	1	—	99	
IXB ..	Lime sulphur	135	16	3	88	**
	Captan	158	5	—	97	

Asterisks denote differences significant at the 5% level (*) or the 1% level (**).

Crop weight results, omitting data from replants, are summarized in Table V. Apart from the significantly greater crop from captan in planting system IIA (the difference in system IIB only just failed to reach significance), there are indications in these planting systems of a residual effect from the 1954 treatments, in favour of captan. A full statistical evaluation is being deferred until the completion of the experiment.

TABLE V.

MEAN CROP WT. (LB. PER TREE) EXCLUDING REPLANTS, SEPTEMBER 27–29, 1955.

Block	Post-blossom fungicide+		Planting system		
	1954	1955	IIA	IIB	IXB
1	LS	LS	20·8	33·6	15·1
	C	C	58·6	68·2	21·6
2	LS	C	45·0	61·6	30·7
	C	LS	22·6	52·8	39·4
3	C	LS	29·3	30·9	19·8
	LS	C	49·9	70·2	41·3
Overall means for 1955 treatments		LS	24·2 *	39·1	24·8
		C	51·2 *	66·7	31·2

* Differ significantly (at the 5% level).
+ LS = Lime sulphur. C = Captan.

Commercial grading was carried out on October 7 and 21 on the bulked crop. Data on fruit size and colour are summarized in Table VI, averaged for all blocks within each planting system. Variation between blocks is so great as to make statistical analyses of little value, and no differences in size, colour or percentage of culls are consistent enough to warrant comment at this stage.

TABLE VI.

GRADING DATA : FRUIT SIZE AND COLOUR.
MEAN PERCENTAGE FIELD CROP IN EACH CATEGORY, OCTOBER, 1955.

Planting system	Post-blossom fungicide (1955)	Total fruit of at least "Fancy" grade	"Fancy" fruit graded for size		Fruit of "Fancy" size, but below standard for colour	Blemish	Culls	Loss in Store
			>2¼ in. diam.	<2¼ in. diam.				
IIA (a)	Lime sulphur	52·2	47·9	4·3	6·0	13·0	22·8	6·0
	Captan	53·4	45·7	7·7	11·8	9·8	18·6	6·4
IIB (a)	Lime sulphur	65·2	46·3	18·9	8·2	7·2	11·7	7·7
	Captan	62·0	43·9	18·1	18·8	6·6	7·8	4·8
IXB (b)	Lime sulphur	59·8	50·2	9·6	9·3	18·2	8·6	4·1
	Captan	63·4	49·9	13·5	13·6	14·7	4·8	3·5

(a) Graded October 21. (b) Graded October 7.

Miscellaneous.

Observations in the course of the season again suggested that Cox spot on leaves was worse on the captan-treated plots but, except on two or three guard trees of the variety Winston, it caused negligible damage on this site.

Discussion.

As mentioned in the previous paper (1) a full statistical evaluation of the effects of the two post-blossom fungicides will not be possible until the end of 1956, the third year of this experiment. Nevertheless, it is possible to make limited comment at this stage, with particular reference to the performance of the two fungicides in a dry season.

As in 1954, an excellent control of apple scab was afforded by both treatments. The substitution of captan for lime sulphur tended to reduce fruit russet, but the extent of russeting was much less than in 1954, and the beneficial effect of captan correspondingly less marked. The use of captan as a post-blossom fungicide in 1955 led to a significant increase in crop from trees in one of the planting systems, and increases which failed to reach significance in the two other systems. Data on crop quality showed no consistent difference between treatments.

From general experience, the limiting factor in the substitution of captan for lime sulphur is probably the increased incidence of apple mildew that often ensues. In the Long Ashton experiments, the very low incidence of the disease on blossom trusses in June 1955 was in marked contrast with the

records obtained in August 1954 from shoots on the same plots following the 1954 captan treatments. This suggests that much of the infection recorded on these shoots in 1954 may have been confined to the leaves, and did not become established in the buds. The records of mildew on the current season's shoots in September 1955 again showed high incidence, except on plots that had received lime sulphur throughout in both seasons. At the time of winter pruning, few obviously mildewed shoots were seen on any plot, indicating that, as in the previous season, much of the shoot infection may have been confined to the leaves, although a full evaluation must await a further recording in the late spring of 1956.

There has been no evidence to date, therefore, to show that, under Long Ashton conditions, and where pre-blossom lime sulphur is applied, the increased level of apple mildew on Cox's Orange Pippin following the substitution of captan for lime sulphur as a post-blossom fungicide is sufficiently high to be of economic importance.

Summary.

In the dry summer of 1955, the second year of a three-year trial, an excellent control of apple scab (caused by *Venturia inaequalis*) on the variety Cox's Orange Pippin, was afforded by post-blossom applications of either captan (0·075%) or lime sulphur (0·75%), following pre-blossom lime sulphur sprays.

There were indications that the substitution of captan for lime sulphur in the post-blossom sprays resulted in a higher crop, with less fruit russet.

The incidence of apple mildew (caused by *Podosphaera leucotricha*) on shoots of the current season's growth was markedly less on the plot receiving lime sulphur throughout both seasons 1954 and 1955. There was no evidence that the attack on other plots was sufficiently heavy to be of economic importance.

Acknowledgments.

The authors wish to thank Mr. E. W. Hobbis and Mr. E. Catlow (for placing the trees at their disposal, and for supplying detailed crop and grading data), Mr. R. W. Marsh (for his continued interest) and Mr. D. H. Payne and Mr. R. T. Burchill (for assistance in recordings).

The meteorological data were kindly supplied by Mr. G. E. Clothier from the Station records.

REFERENCES.

(1) Byrde, R. J. W., Clarke, G. M. and Harper, C. W. (1955). Spraying experiments against apple scab at Long Ashton, 1954. *Ann. Rep. Long Ashton Res. Sta.*, 1954, 147.

(2) Marsh, R. W. (1947). Fruit spraying trials with certain recently-introduced fungicides. *J. hort. Sci.*, **23,** 185.

(3) Pearce, S. C. (1947). The measurement of fruit crops by sampling. *Ann. Rep. E. Malling Res. Sta.*, 1946, 77.

SPRAY APPLICATION PROBLEMS: XXVIII. A COMPRESSED AIR SPRAY NOZZLE FOR LABORATORY AND FIELD EQUIPMENT FOR SMALL-VOLUME AIRFLOW SPRAYING.

By H. G. H. KEARNS and N. G. MORGAN.

Introduction.

The application of small (low) volumes of spray materials of normal* or concentrated strengths requires the atomization of the liquid into small droplets. The droplets must be within a size range that ensures impaction on the target and the number per unit area such that coalescence and run-off are avoided. At the same time the distribution of droplets that satisfies biological requirements must be known. Unfortunately, in many instances, this essential information is not available, and conflicting results are obtained when different types of machinery and techniques are used. The problem is made more complex by variations in the nature of the spray target, particularly in the case of tree crops. Therefore, until more biological data are available the application of small volumes must of necessity be a matter of field experience based on trial and error with a particular machine.

The amount of spray material required for effective deposits is very small, and it is not unusual to apply by hydraulic spraying (run-off) 25 times the volume of liquid that is actually required to ensure effective distribution on the target. The only method of achieving substantial economy is by using much smaller droplets : the smallest size of droplet that is able to reach and impact the target ensures the maximum economy of the spray material.

With some ground crops, such as young cereals, the spray machine can travel directly over them, and the droplets usually have sufficient energy to travel the small distance to the target without further assistance. On tree crops, however, small droplets of less than 200 microns in diameter, however formed, have in most cases insufficient energy to travel the necessary distance, and require assistance to enable them to reach the spray target. This is most conveniently carried out by means of an air current, the volume and velocity of which varies according to the particular circumstances.

Owing to the practical difficulties of obtaining uniform distribution of small droplets by hand-spraying methods, spraying machines used for the application of small volumes should be automatic. The volume of spray liquid per acre applied by an automatic machine is the volume discharged per minute multiplied by the time in minutes taken to spray the acre. Most automatic machines operate at about 2–4 miles per hour, taking about 10–15 minutes per acre, depending on the planting distance of the crop. Experimental work indicates that the volume applied per acre by small volume methods may vary from $\frac{1}{2}$ to 50 gallons, depending upon the crop, the organism to be controlled, and upon the spray material used. Under these conditions, therefore, from $\frac{1}{2}$-pint

* Normal strength refers to the concentration of water-diluted materials normally applied by conventional spray equipment to ensure wetting the spray target as completely as possible. This is accompanied by considerable run-off.

to 5 gallons of spray liquid must be atomized per minute to produce the smallest droplets that will impact on the target.

The atomization of a liquid into droplets, most of which are less than 80 μ diameter, can be achieved by high-speed spinning discs, high-speed air shear, or by compressed air nozzles. The use of the first method is limited to volumes of 5–10 fluid oz. per minute and necessitates costly duplication for larger volumes ; because of the need for high speed electric motors or compressed air turbine motors it also introduces mechanical complications ill-suited to agricultural machinery.

The compressed air nozzle offers a simple solution. Most of the liquid is broken up into small droplets of reasonably uniform size without complicated mechanical difficulties of manufacture or maintenance, provided that the throughput per nozzle is not more than 10 fl. oz. per minute. This type of nozzle consists of a liquid feed tube surrounded by an air annulus. The fluid is shorn from the tip of the feed tube and atomized by the high speed air stream from the annulus. Droplet size can be adjusted by changing the ratio of liquid to air delivery. This can be done either by adjusting liquid feed rate by pressure or valve, by altering the size of the air annulus or by adjusting the air pressure.

The disadvantages of the nozzle are the need to use small apertures for the liquid jet, which is readily blocked unless the filtering system is well designed ; the nozzle is also subject to wear if hard abrasive materials in suspensions are used, and therefore adjustments or replacements may be necessary fairly frequently. Emulsions and clear liquids, especially oils, cause very little wear.

The design of a nozzle suitable for field sprayers and for laboratory equipment is described.

Specification of the Nozzle.

The atomization of liquids by compressed air is commonly used for paint and lacquer spraying but the nozzles are costly. A simple robust design has been evolved which makes manufacture inexpensive yet accurate. The nozzle can be used singly or in multiple for laboratory or field spraying machinery.

The component parts of the nozzle are readily made of brass, bronze or monel metal. Their dimensions are given in Fig. 1 and a section of an assembled nozzle is shown in Plate VII.

The Nozzle Body. This is of round bar 7/8″ diameter and 2″ long ; at one end an air nozzle cap and a liquid nozzle are fitted, each with its own portway. At the other end of the body is a control for a needle valve with a leak-proof gland.

At the air-cap end a free-fit thread $\frac{3}{4}$″ × 26 t.p.i. is cut for $\frac{1}{4}$″, with a 1/16″ wide recess just greater than the depth of the thread. This permits easy and accurate location of the air cap in relation to the liquid nozzle. Concentric to the cap thread the centre of the body is bored for 3/8″ depth and tapped 5/16″ × 26 t.p.i.

The other end of the body is bored 17/64″ for a depth of 15/16″ and then counterbored 29/64″ for a depth of $\frac{3}{8}$″. These bores are tapped 5/16″ and $\frac{1}{2}$″ B.S.B. (26 t.p.i.) to accommodate respectively a gland nut and a needle control screw similarly threaded to give a free fit. The two ends of the centrally bored

portions of the body are made to communicate by drilling a way 3/16″ diameter.

At two directly opposite points on the air-cap end of the body and $\frac{3}{4}$″ below the top, two $\frac{1}{8}$″ diameter holes are drilled. One of these is drilled into the central passage which then forms a conduit to the liquid nozzle. The other hole is drilled for a depth of 3/16″ and into this and parallel to the central passageway in the nozzle, from the cap end, a $\frac{1}{8}$″ diameter hole is bored : this forms a passage way for compressed air into the air cap.

The Air Cap. This is externally $\frac{3}{4}$″ from top to base ; the base is $\frac{7}{8}$″ diameter for $\frac{3}{8}$″ and then changes to 9/16″ diameter ; the step so formed and also the top of the nozzle are chamfered for about 1/16″. Internally the nozzle is accurately bored 7/16″ for a depth of 11/16″, and this ensures concentric location on the liquid nozzle : it is also counterbored $\frac{1}{2}$″ × 1/32″ to form an air passage ring. The wider end of the cap is counterbored for 5/16″ depth for a $\frac{3}{4}$″ B.S.B. (26 t.p.i.) free-fit thread. The smaller end of the cap is then bored to accommodate the liquid nozzle tube and to form an annulus for the air. For most purposes an annulus width of 1/64″ (15 thousandths in.) is adequate for a liquid nozzle of 4/64″ external diameter. The hole in the air cap must be accurately and cleanly bored concentric to the liquid nozzle.

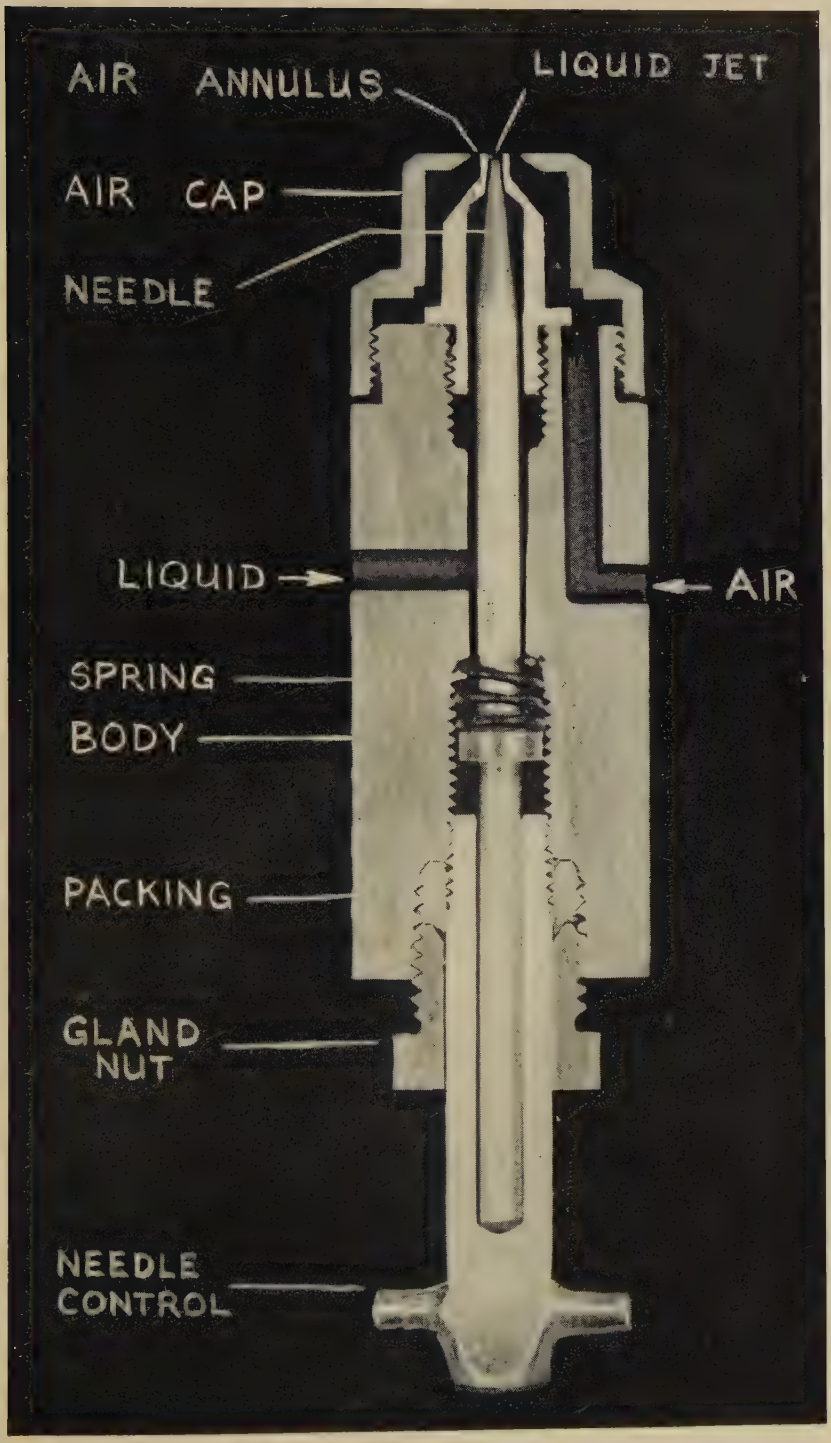
The Liquid Nozzle. This is 7/16″ diameter at the base, for a length of 3/16″ less ·002 in. to make it a push fit into the air cap. A parallel nozzle tube is turned with an overall diameter of 4/64″ × $\frac{1}{8}$″ long ; a chamfer of 45° is turned from the 7/16″ diameter to the base of the 4/64″ tube. The tube of the nozzle is bored either 2/64″ or 3/64″ diameter for a depth of 5/32″. The base of the nozzle, below the 7/16″ diameter, is 5/16″ O.D. × $\frac{1}{4}$″ and threaded 26 t.p.i. free-fit. The base of the nozzle is bored 3/16″ I.D. to meet the bore of the parallel tube. The base of the tube is then taper-reamered to match accurately the taper of the control needle. On two opposite sides of the 7/16″ dimension two flats are milled to give a dimension of 5/16″ ($\frac{1}{8}$″ W. spanner).

The Needle Control Assembly. This is fitted to the base of the nozzle body and consists of a taper needle, a needle control screw and a packing gland nut. The gland nut has a $\frac{1}{8}$″ deep hex. to fit a 5/16″ W. spanner and is turned $\frac{1}{4}$″ long for $\frac{1}{2}$″ 26 t.p.i. and bored centrally 21/64″.

The taper needle is $3\frac{3}{16}$″ long × $\frac{1}{8}$″, one end turned to a 7° taper point ; a $\frac{1}{4}$″ diameter disc 3/32″ thick, bored $\frac{1}{8}$″, is soldered to the needle $1\frac{3}{4}$″ from the tapered point. This acts as a stop for a light spring $\frac{1}{4}$″ O.D. × 5/16″ long which fits over the needle and retracts the needle from the bore of the liquid nozzle when the needle control screw is unscrewed. The needle control screw consists of a round brass rod $1\frac{3}{4}$″ long by 5/16″, threaded one end for $\frac{1}{4}$″ length 26 t.p.i. and bored 5/32″ for a depth of $1\frac{1}{4}$″, to accommodate the parallel end of the needle ; a tommy bar $\frac{5}{8}$″ × $\frac{1}{8}$″ is fitted $\frac{1}{4}$″ from the end of the control screw for ease of adjustment. A short piece of asbestos string packing is fitted beneath the packing nut around the control screw to prevent escape of liquid.

Mounting of the Nozzle.

A convenient way of mounting a single nozzle is to solder or braze on two adaptors with their bases milled to the radius of the body and the other end threaded $\frac{1}{4}$″ B.S.P.M. These adaptors are located opposite each other in line with the air and liquid entry ports. This arrangement permits of the use of



Section of assembled compressed air spray nozzle.

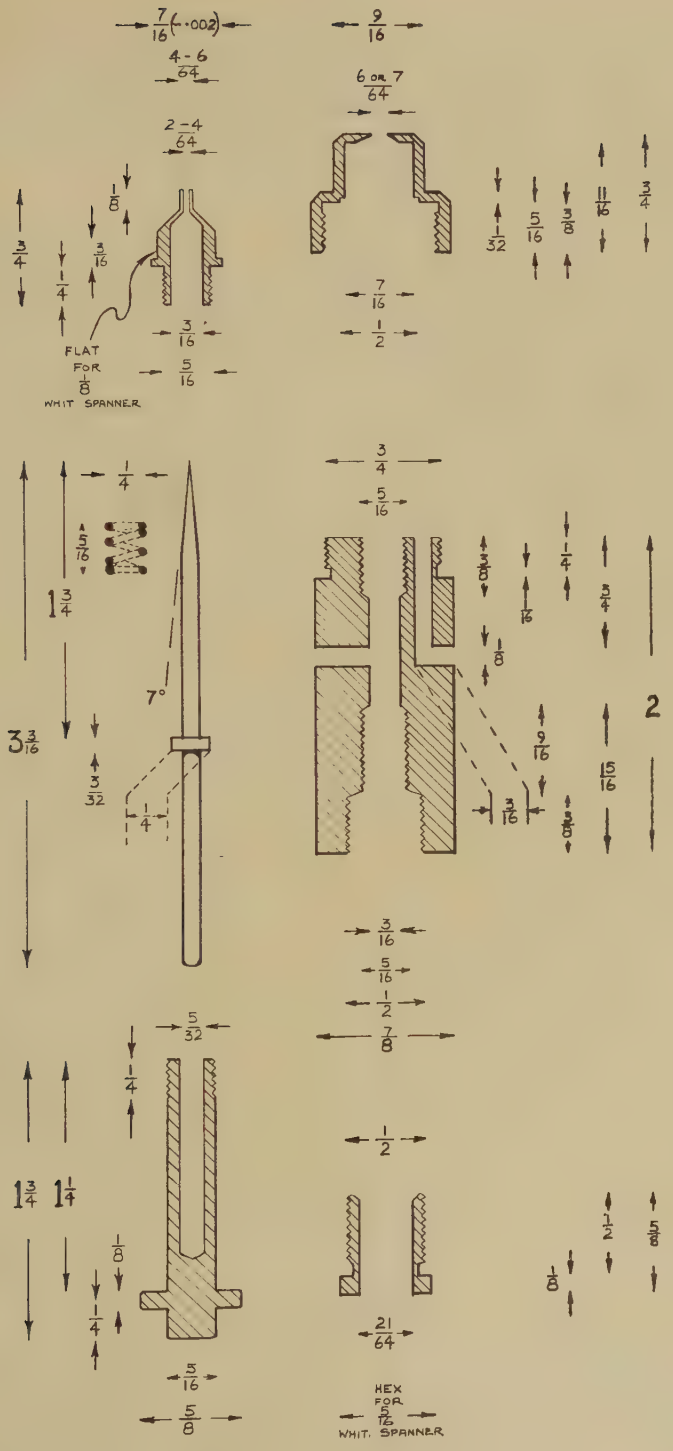


Fig. 1. Components of compressed air spray nozzle.

the nozzle for a wide range of duties including laboratory spraying equipment for atomizing liquids and for droplet formation on air-flow mist sprayers etc. Another method of mounting several nozzles for mist sprayers is to assemble them in the form of a straight manifold : in this instance the nozzles are brazed onto and between two $\frac{1}{2}''$ bore copper tubes, one tube acting as an air line and the other for the liquid (Plate VIII).

Nozzle Adjustment.

The droplet " spectrum " produced by the nozzle can be varied by adjusting the relative throughputs of the liquid and air, the former by means of the taper needle and the latter by the size of the annulus surrounding the tubular portion of the liquid jet. Liquids are atomized by the nozzle with an air flow of 2 c. ft. per minute at a pressure of 15 lb. p.s.i. and above. An increase in air pressure or restriction of liquid flow results in the formation of very fine droplets. The choice of liquid jet bore and air annulus size depends upon the range of droplet sizes required. The following combinations of air caps and liquid jets cover most spraying requirements :—

Liquid nozzle aperture.	*Overall diameter of liquid nozzle tube.*	*Air cap aperture.*
2/64 in.	4/64 in.	6/64 in.
3/64 in.	4/64 in.	6/64 in.
4/64 in.	6/64 in.	7/64 in.

In a number of instances the tapered control needle can be omitted. This simplifies the design of the nozzle, as a packing assembly is not required and it then only becomes necessary to select the appropriate air cap and liquid nozzle to suit the required performance.

SPRAY APPLICATION PROBLEMS: XXIX. A TRACTOR DRIVEN AIRFLOW MIST-BLOWER FOR EXPERIMENTAL WORK.

By H. G. H. KEARNS and N. G. MORGAN.

Introduction.

Mist-blowers are well suited for the application of many synthetic insecticides, the high efficiency and persistence of which make it possible to obtain results from relatively poor distribution. On the other hand the protective fungicides must be applied so that the distribution is uniform and the quantity sufficient on all the surfaces liable to infection ; this is an exacting requirement beyond the capacity of most mist-blowers except on easy targets.

In 1950 a large airflow mist-blower was designed for studies on the application of small volumes of spray materials for crop protection, locust control etc. (1). The volume and pressure of both air and liquid delivered by this machine, as well as the direction of delivery, were adjustable for a wide range of experimental duties, and the machine was necessarily larger than would normally be required for economic use. It was found that spray materials could be applied by mist-drifting or by forceful application of the droplets. In the former instance small droplets are carried by the wind for considerable distances and they fall out on exposed portions of the target ; in the latter, the air delivered by the fan propels droplets to and through the vegetation, often against the prevailing wind. Mist-drifting is used for the application of very small volumes of the order of ½–5 gallons per acre and it is necessary to use small droplets, the size depending upon the performance required. Forceful application is generally associated with the use of larger volumes, aiming at a much more uniform coverage without undue waste by run-off of the spray.

It was found from tests with this machine that a blower with an air delivery of 8,000 -12,000 c. ft. per minute at 40–90 m.p.h. nozzle velocity was a satisfactory compromise of cost, size and performance for mist-drifting and forceful application. A machine of this size required 12–20 h.p. so it was possible to design it as a trailer model which could be hauled and driven by tractor.

A machine has been designed for wheeled tractor haulage and power take-off drive suitable for applying all types of spray materials in small and large volumes with a droplet size adjustable from 25 to 250 microns diameter, depending on the throughput. The machine has many uses for experimental work on temperate and tropical crops and it has so far been used on bush apples and coconuts (Fig. 4).

Brief Specification (Figs. 1 & 2).

The machine comprises a two-wheeled chassis on which are mounted a tank, fan and air trunking, gearbox assembly, hydraulic pump, air compressor, hydraulic and compressed air nozzle assemblies and the associated ancillary components and pipe work. The power is conveyed from the tractor by a telescopic drive shaft.

The Chassis. The chassis is made of 3″ × 2″ channel sections, 7′ 6″ long by 4′ 3″ wide, with triangular corner plates and cross members electrically welded to give rigidity. The cross members are fitted in positions on the chassis to act as supports for the gearbox, fan and tank. A 2″ square axle of 62″ track is fitted at the rear of the chassis, so that the machine is forward-heavy and much of the weight is carried by the tractor rear wheels. One pair of tyres 16 × 6·25 is fitted to the axle. The chassis is fitted with a pressed steel drawbar so that the hydraulic lift and hitch assembly of the Ferguson tractor can be used ; alternative drawbars are available for other makes of tractor. The hydraulic lift and hitch beneath the back axle of the tractor ensures easy attachment and haulage. A large roller is fitted on each side of the drawbar to prevent the rear tyres of the tractor fouling the telescopic power-take-off shaft ; on contact, the noise of the roller warns the tractor driver that he is making an excessively sharp turn.

The Tank. Alternative types of cylindrical tanks are provided, one with a 2″ B.S.P. filler aperture as shown in Fig. 1 for use with oils, and a second type with a 9″ filler hole fitted with a drop-in anti-splash filter basket and cover lid for aqueous sprays. Both tanks are of welded construction of $\frac{1}{8}$″ plate and fitted with four $1\frac{1}{2}$″ B.S.P. threaded flanges for the plumbing and two mild steel foot brackets for easy attachment to the trailer chassis. The tanks are hot galvanised after manufacture : their capacity is 55 gallons and they are readily interchangeable.

The Fan. A centrifugal fan was selected as most suitable for this type of mist-blower as it is of simple design and rugged construction. Large ball races, in a housing mounted on a pedestal base, support the shaft, at one end of which the runner is fitted and at the other a vee pulley for the drive. The fan casing (approximately 34″ × 18″) is constructed of 10G mild steel plate and mounted on a flange bearing on the pedestal so that it can be rotated and locked in any position. This arrangement makes it possible to adjust the fan trunking to deliver vertically or at any angle on either side of the machine, an important feature when spraying crops of various heights. The diameter of the casing and the design of the bearings of the drive shaft are such that three alternative sizes of runners can be fitted according to the horse power available from the tractor ; the greater the width the greater the horse power required. The diameter of the runner is 24″ and the widths 7″, 9″ and 11″, each with its correct size of guide vane assembly. The speed of rotation is 2,000 r.p.m. The 7″ wide runner requires about 12 h.p., so that it is suitable for tractors of about 23/25 h.p., the surplus power being necessary mainly for haulage but also for driving ancillary equipment. A stout steel mesh guard is fitted over the air inlet port.

The Air Nozzle. The size of the air delivery aperture determines the velocity of the outflowing air. A decrease in size of the aperture results in an increase in air velocity, the maximum velocity depending upon the design of the fan. The shape and construction of the delivery trunking determine the general " contour " of the air stream. For a particular horse-power, air volume is considerably more important for the distribution of droplets than air velocity, and a velocity of 50–60 m.p.h. is adequate for most purposes provided the volume is sufficient. A general-purpose air nozzle for this fan has a circular

aperture of 17″ diameter. The rectangular base of the trunking is 24″ × 20″ and tapers for 17″ to a circular collar with parallel sides 4½″ deep. The collar is used for the attachment of the spray nozzles. With this assembly the fan delivers 8,000 c. ft. of air per min. at a nozzle velocity of 60 m.p.h.

The Drive Assembly. The speed of a tractor power-take-off drive is approximately 425 r.p.m. The power from the tractor is transmitted by means of a telescopic shaft fitted with universal joints to a gearbox mounted on the trailer. The fan is driven from the gearbox by a vee belt drive.

The telescopic shaft is a Hardy Spicer heavy duty type No. 1412, which provides a substantial margin of safety. The totally enclosed sump-lubricated gearbox is a single reduction helical gear unit with an increasing speed ratio of 1 to 4, and it is capable of continuous duty. The output shaft is fitted with an 8″ diameter 6-groove vee pulley size B and a 6″ 3-groove vee pulley size A. The 8″ pulley drives the fan through an 8″ pulley at 17″ centres and the 6″ pulley drives an air compressor through an 8″ pulley at 21″ centres. A 4″ single vee pulley size B is also fitted to the fan shaft to drive a hydraulic pump. Endless vee-belts are used on all except the pump drive, which is a Brammer split link. The pulleys are of cast iron or aluminium alloy, and belt tension is adjusted by movement of the gearbox and compressor. The drive shaft of the gearbox is in line with the drawbar to ensure correct alignment of the telescopic shaft. All the belt drives are guarded by a single readily detachable shield.

The Air Compressor. A single stage two cylinder compressor, lubricated by pump and splash from an oil sump, and delivering 11 c. ft. of air at 100 lb. p.s.i. is fitted. The compressed air is passed through a flexible steel pipe to an air receiver fitted with a relief valve, pressure reducing valve, pressure gauge, draincock and take-off valve. The air is conveyed by means of oil resistant hose to a compressed air nozzle assembly.

The Hydraulic Pump. A ¾″ phosphor bronze gear pump driven by the Brammer belt drive is capable of delivering 10 g.p.m. at 75 lb. p.s.i. It is fitted with an adjustable relief valve. The pump is used for agitation of the spray liquid in the tank, for delivering the liquid to the nozzle assembly, and it can also be used to fill the tank from an outside source.

The pipe circuit is fitted with control valves so that spray liquid can be pumped either from the large tank fixed to the chassis or from small containers for special tests. All liquid entering the pump from whatever source first passes through a large, easily cleaned filter fitted with a 40 mesh monel metal gauge element. The piping is fitted at suitable points with unions for easy dismantling and with plastic pipe sections to prevent fractures from vibration. The delivery line from the pump to the nozzle assembly is of oil-resistant hose. A pressure gauge is fitted to the circuit for a short period only, as a test instrument to adjust the relief valve ; it is then removed. This procedure avoids damage to the gauge from vibration and pressure fluctuations.

The Nozzle Assemblies. The spray liquid is atomized either by compressed air nozzles described in this Report (2) or by hydraulic swirl-plate nozzles. The former are intended for the atomization of small volumes of fluid into small droplets of less than 80 microns in diameter ; the hydraulic nozzles produce droplets from 80 microns diameter upwards, according to the fluid used and the swirl plate and disc assembly.

The Compressed Air Nozzle Assembly (see Plate VIII, Fig. 1). Four compressed air nozzles are assembled and brazed in line at 3″ apart between two half-inch bore copper tubes to form a manifold : one tube conveys the liquid and the other the air to the nozzles. Each end of each tube is fitted with a threaded bush with lock nuts. This arrangement makes a simple and efficient method of mounting the assembly in the mouth of the air delivery trunking. One end of each tube is fitted with a blank cap and the other with the hose from the hydraulic pump or air compressor. The flow of liquid to the nozzles is adjusted by the pressure and by fitting a restrictor disc with $1\frac{1}{2}$, 2, $2\frac{1}{2}$, 3 or 4/64 in. diameter holes in the hose circuit. By adjustment of the nozzle liquid-jet valves and the air flow and pressure, it is possible to obtain a range of throughputs and droplet sizes. The performance will vary with the physical nature of the fluid and can be ascertained only by trial (p. 150).

The Hydraulic Nozzle Assembly (Fig. 3). A $\frac{1}{2}$″ bore copper tube is fitted with four pairs of small volume nozzles (3) spaced at $2\frac{1}{4}$″. The nozzles in each pair are at 90° and each has its own easily cleaned filter unit. Each end of the tube is bushed and fitted with lock nuts and locating plates so that the assembly fits into the same apertures used for the compressed air nozzle assembly. One end of the tube is fitted with a blank cap and the other with a hose line attached to the pump delivery circuit.

The delivery hose pipe line from the pump is fitted with a filter unit with a 100 mesh monel metal element which prevents blocking of either the compressed air or the hydraulic nozzles.

Operation of the Machine.

The machine is intended to be used mobile and the volume of liquid released per acre is dependent on the time taken to traverse an acre and the throughput of the nozzle assembly. It is therefore important to fit to the tractor an accurate low speed speedometer, as much of the spraying will be carried out at $1\frac{1}{2}$–4 m.p.h. The throughput of the nozzles must be calibrated carefully with the nozzles operating in the fan air stream. This is conveniently carried out by using the pump circuit and a small container with a known volume of fluid.

The choice of method of atomization will depend on the droplet size requirement : for many purposes the hydraulic nozzle will be found satisfactory. One to eight nozzles may be used, those not required being blocked by fitting undrilled discs. The flow pressure should be 75 lb. p.s.i.

The setting of the position of the fan delivery trunking will vary according to the nature of the target and the spray deposit required. For example, when spraying tall trees, such as coconuts, with oil formulations, the best results are obtained by mist-drifting with the air trunking set vertically.

If a much better coverage is required, for example on a bush fruit crop, it is necessary to use the velocity of the air to carry the droplets through the vegetation and in this instance the distance between the machine and target would be only a matter of a few yards.

The effectiveness of mist-blowers is greatly influenced by the velocity of the wind, and a wind of six or more miles per hour can greatly affect performance. Under some circumstances the wind can be used to advantage, more

Fig. 1. Near-side of machine showing the compressor and the air and liquid feed lines to the compressed air nozzle assembly.

Fig. 2. Off-side of machine showing the gear-box, fan drive and hydraulic pump assembly.

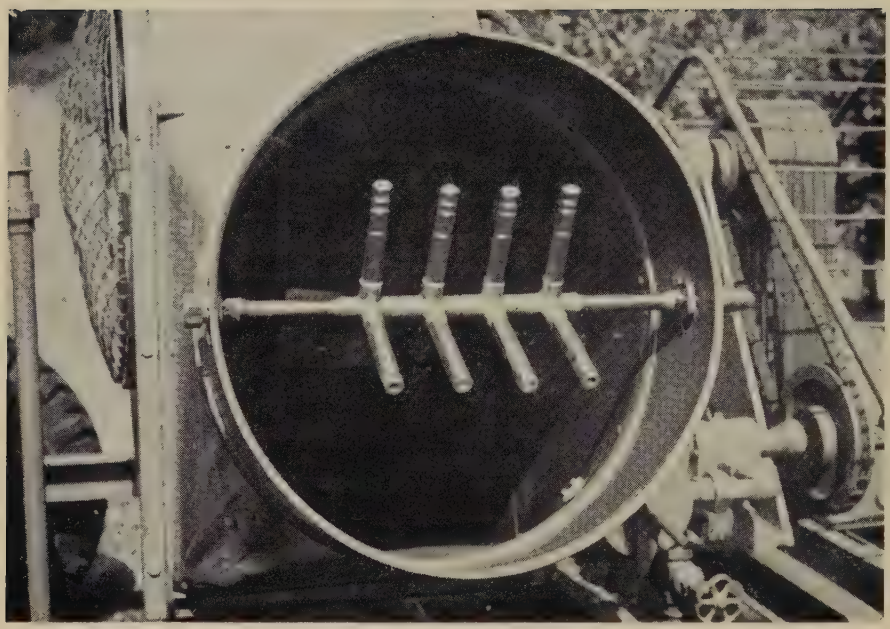


Fig. 3. The hydraulic nozzle assembly mounted in the air delivery trunking.

Fig. 4. The mist-blower in a coconut plantation.

especially with mist-drifting if the direction of the wind remains reasonably constant. Under windy conditions it is often necessary to use a light cab on the tractor to protect the driver from spray deposits.

REFERENCES.

(1) H. G. H. Kearns and N. G. Morgan (1951). An experimental " air-flow " small volume sprayer and duster : abridged specification. *Ann. Rep. Long Ashton Res. Sta.*, 1950, 132–137.

(2) H. G. H. Kearns and N. G. Morgan (1956). Spray application problems : XXVIII. A compressed air spray nozzle for laboratory and field equipment on small volume air-flow spraying. *Ann. Rep. Long Ashton Res. Sta.*, 1955, 146–150.

(3) H. G. H. Kearns and N. G. Morgan (1950). A lightweight spray nozzle ($\frac{1}{4}$° B.S.P.) for " small " and " large " volume spraying. *Ann. Rep. Long Ashton Res. Sta.*, 1949, 111–114.

SPRAY APPLICATION PROBLEMS: XXX. MOBILE OBSERVATION PLATFORMS FOR THE EXAMINATION OF TREE CROPS.

By H. G. H. KEARNS and N. G. MORGAN.

Among the many operations necessary for the field evaluation of spray application techniques, the following may give rise to difficulties unless special observation equipment is available :—

(1) Assessments of infestation by insect pests or infection by diseases.
(2) The sampling of foliage and other parts of the plant for analysis of spray deposits.
(3) Observations on the effect of spray applications on the movement of foliage and on the behaviour of insects.
(4) The taking of photographs *in situ* for recording and illustrative purposes.

These operations present few difficulties on crops under 7 ft. in height. Although with tall fruit trees much can be done on the branches below 7 ft., an accurate assessment of the efficiency of spray application techniques is not possible unless a complete survey is made over the whole tree. At 8 ft. or more above ground tripod picking-ladders or rung ladders have to be used and the frequent shifting of these to different positions is time-consuming and often does not allow of satisfactory observations. A more convenient and rapid procedure is required, particularly when large numbers of samples have to be taken in a short time.

Some tropical tree crops present even greater difficulties. With coconuts, for example, observations are required on foliage, flowers and fruit which may be as much as 60–70 ft. above ground. Mobile platforms are needed to enable trained observers to reach the required sites easily, quickly and in safety.

Tractor-mounted equipment for trees up to 18 ft. high.

The equipment so far used on fruit trees for the type of work described by Morgan and Martin (1) is a modified hydraulic loader fitted to a Ferguson tractor (Figs. 1 and 2).

Two rams operated from the hydraulic system of the tractor are used to raise and lower the curved tubular steel beams on which the loading bucket is hinged, and a third ram is normally used for tipping the bucket.

The bucket was converted to an observation platform by fitting a rigid tubular steel guard rail at a height of 4 ft. from the base. With the beams fully raised the platform base is 11 ft. from the ground and enables an observer of normal height to reach branches up to 18 ft. (Fig. 1). Steel side-plates were also fitted outside the bucket, affording a foot-hold for the observer when reaching branches on either side. A removable metal tray for holding specimens, sampling and other equipment, was fitted inside the bucket. On the guard rails a rigid universally adjustable camera support can be fitted in appropriate positions.

Fig. 1. The tractor-mounted observation platform in use.

Fig. 2. The modified loading bucket showing guard rail, side-plate foot-rest and sample container.

Fig. 3. Self-supporting ladder in use in a coconut plantation.

With the bucket at ground level the observer stands inside the guard rail and the operations necessary to raise the beams are carried out by the tractor driver who periodically levels the platform with the third ram. When the required height has been reached non-return valves are used to maintain the three rams in position. At its maximum height the platform projects 3 ft. forward of the front wheels of the tractor which can then be driven into position.

A 2 cwt. concrete block is carried on the tractor tow-bar, to reduce the extra weight imposed on the front axle, and the tractor wheels are set at least 60 inches between centres for increased stability and safety. Samples can be taken over a tree area of approximately 12 ft. square from one position of the platform. In open plantations the tractor can be driven from tree to tree without lowering the platform. The necessity for periodic levelling of the platform as it is being raised can be eliminated by the replacement of the third ram by parallel-linked arms so fitted as to maintain the platform in the horizontal position irrespective of the height of the beams. This equipment forms the basis for a mobile observation platform and further modifications may extend its use.

Extending, self-supporting ladder for trees up to 72 ft. high.

Equipment of this type (Fig. 3) was obtained from Germany and has been used for observation and sampling on coconut palms in Zanzibar and the Solomon Islands.

It consists of a rigidly constructed steel telescopic ladder in three sections mounted on a wide-based steel chassis for haulage by hand, truck or tractor. The trailer chassis also forms an integral mounting for the self-supporting ladder when extended. The adjustments for the extension of the ladder, its plumbing and inclination, are operated through gears and automatic brakes ; two operators can easily erect and lower the ladder. On rough and uneven ground it is necessary to move the ladder by a tractor or truck and then manhandle it into field position ; this may require a team of six men.

Before elevation the trailer is located on firm ground, and the clamps holding the ladders to the trailer chassis are released. The ladder is partially elevated by a winding mechanism fitted with a self-acting cone brake and spring-loaded safety pawls. Partial elevation causes the trailer to rest on the axle wheels and on a centrally mounted double rubber-tyred castor wheel. The ladder position is then adjusted so that when fully erected it will be in line with the target to be reached. The ladder is extended to the required height and is then carefully adjusted by means of a plumbing device so that it is absolutely vertical. If its lateral position is incorrect it is moved, after lowering to about 25 ft., by means of the road wheels. The ladder is then elevated at an inclination, shown on the inclinometer table, suitable for the height. The brakes are applied to the road wheels, four stabilising jacks are lowered to rest firmly on the ground, and finally the ladder sections are adjusted so that they are held firmly by safety spring pawls. It is particularly important that the wide disc bases of the stabilising jacks should rest on firm soil, as subsidence of the soil, due to hidden ant or termite cavities, for example, may render the ladder unstable. If the fully extended ladder is used in a wind of 10 or more m.p.h. it is desirable to use guy ropes, one on either side of it, held

firmly by men who appreciate the need of taking up movement out of the vertical.

The top section of the ladder is fitted with an observation platform that will extend 4 ft. beyond the ladder and is made with side and end safety rails. It must be retracted before the ladder is lowered. The ladder sections are fitted with deep side rails which give a feeling of security and the treads are square and fitted with non-slip rubber.

On no account should the ladder be moved while fully elevated, nor should it be moved or elevated with a man on it. The total weight of the ladder and trailer is 30 cwt. and at 75 foot elevation at 78° inclination it will support 6 cwt. at the top, but less when the observation platform is extended.

The ladder is normally self-supporting and must on no account be rested against objects, such as trees, which are liable to move. The platform plays a useful part in enabling the observer to reach most parts of the tree.

REFERENCE.

(1) Morgan, N. G. and Martin, J. T. (1955). Spray application problems : XV. Copper and DDT deposits from large and small volume application. *Ann. Rep. Long Ashton Res. Sta.*, 1954, 97.

ACID CHANGES IN CIDER FERMENTATIONS: I. THE SIGNIFICANCE OF ACID CHANGES IN CIDER-MAKING.

By F. W. BEECH and A. POLLARD.

Introduction.

The flavour of a cider depends to a great extent upon its sugar-acid-tannin balance, and if this is unsatisfactory the finer points of quality may be obscured. The degree of sweetness can be controlled by arresting the fermentation or by the addition of sugar at a later stage of cider-making. The amount of tannin stays fairly constant throughout fermentation and is determined mainly by fruit variety. The type of fruit used also affects the acidity of the juice but it is by no means the only factor determining the acidity of the cider, for this can change during fermentation and storage. Aerobic changes leading to acetification are well known and can be controlled by the exclusion of air. These precautions do not, however, prevent other extensive anaerobic changes that can occur during fermentation and storage and that affect the organic acid content of the cider. The advisory work of the Station in recent years shows that such changes in acidity are a frequent source of trouble to cider-makers and, although some of the factors involved have been discussed in recent articles in these Reports and elsewhere, it may be useful to consider their practical significance in more detail.

Acidity and pH.

The cider-maker usually estimates the acidity of a juice or cider by the amount of titratable acid it contains and, so far as flavour is concerned, this works well in practice. Acidity figures do not, however, always explain the behaviour of a cider, and the use of pH values has some advantages, for the pH is a measure of acid reaction and is a main factor determining chemical and micro-biological changes. (For those unfamiliar with the term, the lower the pH, the higher the acidity; being a logarithmic function, pH 3 is ten times more acid than pH 4 : pH 7 is neutral).

The pH of a juice depends upon the ratio of base (ash components) to acid present and, although both can vary, the acid varies to the greater extent between one juice and another. The nature of the acid also affects the pH and this is another cause of variation between the pH value of a juice and the ciders made from it. The amount of acid neutralized by base in a juice varies from about 0·2% to 0·35% (as malic acid) but the amount of free titratable acid can vary from less than 0·2% in the sweets and bittersweets to over 1% in sharp varieties (1). Thus the pH varies from above 4·0 in low-acid juices to about 3·1 in those of high acidity. In sharp varieties most of the acid is malic acid, and the pH and acidity figures are closely related. In low-acid juices the proportion of malic acid is lower and the mixture of acids present is more complex : consequently, the relationship is less clearly defined, and juices with the same titratable acidity can show differing pH values and vice versa.

The Effect of pH on Acidity Changes.

The pH values of apple juices fall into two main ranges : the sharps between pH 3·1 and 3·4, and the sweets and bittersweets between pH 3·8 and 4·2. A few varieties fall into a middle range : bittersharps such as Kingston Black have pH values 3·5–3·6. In a natural cider fermentation the yeasts are relatively unaffected by these variations in pH ; the lactic acid bacteria, on the other hand, are greatly affected by juice pH and their development is favoured by the higher pH ranges.

The yeasts are responsible for only small increases in acidity due to their production of succinic acid. The bacteria, however, can produce marked changes in acidity, the type of change varying from one cider to another. The bacteria normally break down malic acid to lactic acid and carbon dioxide, giving a reduction in titratable acid. They can, however, also produce lactic acid and acetic acid from sugars, including some sugars not fermented by yeasts. This leads to an increase in acidity that will be especially marked if bacterial action begins before yeast fermentation is complete, as may well happen in low-acid juices. As will be shown later, in such juices some malic acid may also be converted into succinic acid instead of the weaker lactic acid ; the general tendency is thus for an increase in acidity, rather than a decline, during the fermentation and storage of ciders from low-acid juices. These various transformations can go on simultaneously and juices of intermediate acidity may rise or fall in acid or give ciders that appear unchanged in acid content although the nature of the acids present may be very different. Juices of high acidity offer a less favourable medium for the growth of bacteria and these may only develop during prolonged storage of the cider : the main change will then be a fall in acid due to malo-lactic breakdown. If such high acid ciders are blended with ciders of lower acidity, conditions become more favourable for bacterial growth and the acidity of the blend will usually fall after a short period of storage. If the blend was originally balanced in composition this loss of acid may entail the addition of further acid at a later stage.

These general trends can be seen from the records of vintage trials of single varieties made in past years at Long Ashton. Ciders made by natural fermentation and conditioned in bottle have shown acid changes that show a definite relationship with juice acidity and pH. Very acid juices (pH 3·1–3·3) have usually shown little change apart from a slight rise in pH. Juices of intermediate acidity such as Kingston Black (pH 3·5–3·6) have gained or lost acid but the pH has not changed greatly, being more stable over this region. Low-acid juices (pH 3·8–4·0) have gained or lost acid in an arbitrary manner with a corresponding shift of pH, while juices of even lower acidity (pH values above 4·0) have invariably become more acid during fermentation and storage.

These acidity changes are discussed more fully in the later articles in this series which describe experiments designed to bring out more clearly the various factors concerned and their relation to cider-making methods. The present article is intended to serve as an introduction and to emphasize points of particular interest to the cider-maker.

Acid Changes and Cider Quality.

Some acid changes in cider fermentations have no deleterious effect upon cider quality : a malo-lactic fermentation, for instance, improves the balance

of a cider made from high-acid juice. But in ciders of medium or low initial acidity such a loss of acid is undesirable and, if it occurs during yeast fermentation, may make the cider vulnerable to sickness. An increase in acidity due to the lactic fermentation of sugars is equally undesirable for it can be accompanied by the production of acetic acid and lead to taints in spite of precautions against aerobic acetification. This is liable to be most serious in low-acid juices where conditions are favourable for the development of bacteria while sugar is still present. Bacterial growth is, moreover, encouraged by the products of yeast autolysis where the ciders are left on the lees after fermentation. In juices of high acidity bacterial development is not likely to occur until yeast fermentation is complete, when the dangers of acetic acid formation are reduced.

The Use of Sulphur Dioxide in Cider-making.

Sulphiting provides the simplest method for controlling cider acidity. As pointed out in an earlier Report (2) and described more fully later (see p. 169), the addition of sulphur dioxide to low-acid juices at the rate of 150 p.p.m. effectively suppresses lactic acid bacteria, and malo-lactic changes do not occur. The overall effect is an increase in acidity during fermentation equivalent to 0·05 to 0·15% (as malic acid). In the fermentation of low-acid juices this increase in acidity, together with the prevention of malo-lactic change, may give a valuable protection against sickness.

The addition of sulphur dioxide at this rate to a freshly pressed juice has the further advantage of giving a more efficient yeast fermentation. Sulphiting the juice not only destroys lactic acid bacteria, including those species responsible for oiliness; it also suppresses acetic bacteria and many non-fermenting wild yeast species. After an initial lag period that usually lasts from 4 to 14 days, the fermenting yeasts grow up sufficiently to bring about a rapid fermentation. In our own tests we have found that such sulphited fermentations then proceed more rapidly and completely than do those in the corresponding unsulphited juices. They give a clean yeast crop, sometimes almost a pure culture of one species that can, if necessary, be used for yeasting a second fermentation. The ciders made from sulphited fermentations of low-acid juices have invariably been of better quality than the untreated control ciders, and off-flavours, that seem to be associated with the presence of certain wild yeasts or bacteria, have been absent.

The addition of SO_2 at a lower level of 100 p.p.m. gives some of these advantages: the fermenting yeasts are encouraged and oiliness bacteria are suppressed, but many lactic organisms survive and develop later in the fermentation (see p. 169). Juices originally low in acid are thus not protected against acid loss during storage. In high-acid juices with pH values near 3·0 the pH restricts bacterial development during fermentation and such juices are inherently less susceptible to deterioration in the early stages of cider-making, for bacterial growth is delayed until all the sugar has been fermented. The main advantage of sulphiting such juices is that it gives a better yeast fermentation. Where ciders are made from blends containing a high proportion of sharp cull fruit, and malo-lactic changes are required in the cider, the advantages of sulphiting at the lower rate must be balanced against the disadvantages of some delay in the onset of acid reduction during storage.

Conclusions.

It will be seen from the foregoing discussion that bacteria can bring about extensive changes in the composition of ciders. In ciders made from fruit high in acid the breakdown of malic acid by bacteria is beneficial and improves the flavour balance. In blends of high and low acid ciders this loss of malic acid can be undesirable if the original blend were balanced in composition. In the fermentation of low-acid juices the effects of bacterial action are more variable and may result in a gain or loss of acidity : moreover, as shown in the later sections, these changes are usually associated with a deterioration in cider quality.

Bacterial fermentations in low-acid juices can be effectively suppressed by sulphiting ; acid changes are prevented and the quality and stability of the cider are improved. This applies particularly to the juices of sweet and bittersweet varieties, but marked improvements have been noted following the sulphiting of juices from fruit as acid as dessert varieties. The cider-maker who adopts the practice of sulphiting must, however, be prepared to find his ciders more acid than in the past and he may need to modify his fruit-buying programme accordingly. If he is obliged to use a fair proportion of acid varieties in his blends, only the low-acid juices should be sulphited at the higher rate of 150 p.p.m. The high acidity of juices from culinary varieties or sharp cider apples gives them some protection against bacterial action and sulphiting is less necessary ; if used, the lower rate of addition (100 p.p.m.) should not be exceeded if some fall in acidity is needed during storage.

REFERENCES.

(1) Phillips, J. D. (the late), Pollard, A. and Whiting, G. C. (1956). *J. Sci. Fd & Agric.*, **7**, 31.

(2) Beech, F. W. and Pollard, A. (1954). *Ann. Rep. Long Ashton Res. Sta.*, 1953, 171.

ACID CHANGES IN CIDER FERMENTATIONS : II. THE EFFECT OF JUICE COMPOSITION.

By J. G. CARR and G. C. WHITING.

Introduction.

The pH of a juice plays an important part in fermentation : it controls not only the growth but also the metabolism of the micro-organisms which may be present. While over the pH range of cider fermentations the metabolism of organic acids by yeasts may not vary greatly, relatively small changes in this range have a considerable effect on the bacterial metabolism of organic acids.

The main organic acid in all apple juices is malic acid. Bacterial fermentation of this acid was observed many years ago, but little work on this topic has been done on English ciders until recently. Carr (1) reviewed earlier work and in his own work paid particular regard to microbiological aspects : malic fermentation was thought to occur widely in English ciders and the use of pH adjustment as a possible method of control was suggested. Steuart (2) drew attention to the possible roles of hetero-fermentative lactobacilli and suggested that acidity might control malo-lactic fermentation by some species. Marshall (3) observed changes in titratable acidity and malic acid content during fermentation and suggested a malo-lactic index as a measure of spoilage by malo-lactic bacteria : he assumed that malic acid was quantitatively converted to lactic acid. The work of Williams (4) and of Phillips, Pollard and Whiting (5) indicated that in some fermentations succinic acid may be the end-product of malic acid metabolism, with or without simultaneous formation of lactic acid. The present work shows that not only the time of onset of attack on malic acid, but also the nature of the end-products is affected by pH.

The work described here is divided into three sections. The first reports the effect of pH on the malo-lactic fermentation under normal cider-making conditions ; the second describes microbiological and chemical changes in two juices suspected to have a high bacterial population in the very early stages of fermentation ; and the third describes the growth of lactobacilli, isolated from ciders, on a medium containing malic acid, with particular reference to the effect of pH on end-products.

Experimental.

1. *Effect of pH on the malo-lactic fermentation.*

Two juices of very different pH were used, one from Sweet Alford (pH 3·9) the other from Bramley's Seedling (pH 3·1). Samples of each were adjusted to the pH of the other, and also to a pH intermediate between the two, by addition of hydrochloric acid or sodium hydroxide solution. The following six samples were fermented :—

Sweet Alford	A	pH 3·91*
,, ,,	B	pH 3·35
,, ,,	C	pH 3·10
Bramley's Seedling	A	pH 4·00
,, ,,	B	pH 3·35
,, ,,	C	pH 3·14*

* natural pH.

TABLE I.

ACID CHANGES DURING THE FERMENTATION AND STORAGE OF SWEET ALFORD JUICES.

Sample	pH	% Acid as Malic acid		% Malic acid	% Lactic acid	% Succinic acid	% Volatile acid as Acetic acid
		Titratable acid	Total acid				
Juice A S.G. 1·047	3·91	0·20	0·38	0·27	Nil	Nil	0·006
Cider A S.G. 1·021	3·86	0·23	0·42	0·21	0·03	0·04	0·008
„ A S.G. 1·001	—	0·25	0·48	0·02	0·17	0·07	0·052
„ A 1 month on lees	—	—	0·57	Trace	0·17	0·07	0·139
„ A 2 months on lees	—	—	0·60	Nil	0·17	0·07	0·162
„ A 3 months on lees	—	—	0·61	Trace	0·17	0·08	0·173
„ A 5 months on lees	3·96	0·41	0·63	0·01	0·17	0·08	0·198
Juice B S.G. 1·047	3·35	0·27	0·44	0·27	Nil	Nil	—
Cider B S.G. 1·021	3·38	0·35	0·53	0·25	Nil	0·04	0·004
„ B S.G. 1·001	3·50	0·45	0·62	0·24	Trace	0·06	0·006
„ B 1 month on lees	—	—	0·57	0·11	0·06	0·07	0·024
„ B 2 months on lees	—	—	0·59	Trace	0·12	0·10	0·062
„ B 3 months on lees	—	—	0·59	Trace	0·17	0·11	0·076
„ B 5 months on lees	3·80	0·35	0·59	Trace	0·17	0·11	0·088
Juice C S.G. 1·047	3·10	0·33	0·50	0·27	Nil	Nil	—
Cider C S.G. 1·021	2·97	0·37	0·56	0·25	Nil	0·04	0·006
„ C S.G. 1·001	3·03	0·41	0·61	0·24	Nil	0·05	0·006
„ C 1 month on lees	—	—	0·62	0·20	Trace	0·06	0·010
„ C 2 months on lees	—	—	0·65	0·20	Trace	0·06	0·010
„ C 3 months on lees	—	—	0·66	0·20	Trace	0·08	0·010
„ C 5 months on lees	3·08	0·41	0·65	0·20	Trace	0·09	0·010

Each sample was sterile filtered into a sterile jar and inoculated with a mixed suspension of *Saccharomyces* sp. and a Gram-positive, catalase-positive homofermentative lactic acid bacterium, isolated from apple juice and capable of carrying out a malo-lactic fermentation. The jars were incubated at 15°C. and samples were taken immediately after inoculation; at S.G. 1·025; at dryness; and after monthly intervals of storage on the lees. Determinations of titratable acid, total acid, volatile acids, malic, lactic and succinic acids were made on each sample by methods previously described (5).

Sweet Alford. The results are shown in Table I.

In the natural juice (A), malo-lactic fermentation commenced before a gravity of 1·021 had been reached and was practically complete at dryness. During storage on the lees no further production of lactic acid occurred.

The juice of intermediate pH value (B) showed no malo-lactic change until after dryness had been reached, but after two months' storage it was complete. The pH of the cider was 0·45 units higher than that of the juice.

The sample adjusted to pH 3·1 (C) showed no more than a trace of lactic acid even after several months' storage, and the pH remained unchanged.

Bramley. The results are shown in Table II.

The juice at the natural pH (C) showed no malo-lactic change even on prolonged storage, and the pH was unchanged.

At the intermediate pH (B) no malo-lactic change occurred until after dryness had been reached; it was almost completed after one month's storage. The pH of the cider was 0·54 units higher than that of the juice.

In the juice adjusted to pH 4·00 (A), malo-lactic change was almost completed between S.G. 1·025 and dryness. The pH of the cider was 0·56 units higher than that of the juice.

No great variations in succinic acid production by the yeast were observed between the three samples of either series. The amounts of acetic acid observed in this experiment were higher than expected from a homofermentative lactic acid bacterium.

2. *Acid changes before fermentation.*

Two Sweet Alford juices thought to have a high bacterial population were incubated at 15°C. and samples were taken daily for determinations as above. Counts of yeasts, acetic acid bacteria and lactic acid bacteria by a plate dilution method were made on one juice after four days' incubation. Acetic acid bacteria and lactic acid bacteria were differentiated by means of the catalase test and Gram stain.

Both juices showed complete or almost complete fermentation of malic acid four days after pressing. In one juice (from Gloucester) malo-lactic fermentation occurred, while in the other juice (from Worcester) malic acid was converted to succinic acid. During the four days' incubation of the latter juice, yeasts increased in numbers from 91,200 per ml. to 340,000 per ml.; acetic acid bacteria from 424 to 336,000 per ml.; and lactic acid bacteria from 400 to 4,000 per ml.

3. *Malo-lactic and malo-succinic changes.*

(i) Numerous isolates of lactobacilli from cider fermentations were inoculated into a medium containing 0·25% malic acid, casein hydrolysate, yeast

TABLE II.

ACID CHANGES DURING THE FERMENTATION AND STORAGE OF BRAMLEY'S SEEDLING JUICES.

Sample	pH	% Acid as Malic acid		% Malic acid	% Lactic acid	% Succinic acid	% Volatile acid as Acetic acid
		Titratable acid	Total acid				
Juice A S.G. 1·043	4·00	0·55	—	—	—	—	—
Cider A S.G. 1·025	4·01	0·54	1·08	0·94	Trace	0·01	0·006
,, A S.G. 1·001	4·52	0·22	0·77	0·02	0·45	0·05	0·065
,, A 1 month on lees	—	—	0·77	Trace	0·53	0·05	0·085
,, A 2 months on lees	—	—	0·82	Trace	0·63	0·06	0·094
,, A 4 months on lees	4·57	0·23	0·80	Trace	0·63	0·07	0·114
Juice B S.G. 1·043	3·35	0·82	—	—	—	—	—
Cider B S.G. 1·025	3·38	0·80	1·10	0·97	Nil	0·01	0·007
,, B S.G. 1·001	3·49	0·77	1·07	0·94	Trace	0·02	0·009
,, B 1 month on lees	—	—	0·71	0·05	0·45	0·05	0·025
,, B 2 months on lees	—	—	0·72	Trace	0·58	0·05	0·054
,, B 4 months on lees	3·89	0·39	0·72	Trace	0·58	0·06	0·066
Juice C S.G. 1·043	3·14	0·94	1·13	0·97	Nil	Nil	0·005
Cider C S.G. 1·025	3·09	0·93	1·08	0·97	Nil	0·01	0·006
,, C S.G. 1·001	3·17	0·92	1·08	0·95	Nil	0·02	0·006
,, C 1 month on lees	—	—	1·11	0·94	Nil	0·03	0·007
,, C 2 months on lees	—	—	1·14	0·93	Nil	0·03	0·007
,, C 4 months on lees	3·16	0·89	1·10	0·93	Trace	0·04	0·006

extract and mineral salts, adjusted to pH 4·2. After incubation the medium was examined for non-volatile acid end-products by means of paper chromatography. Malic acid was found to be completely metabolised. Most of the lactobacilli formed lactic acid with or without a trace of succinic acid, while a few yielded the two acids in almost equal amounts.

(ii) Two closely related heterofermentative cider lactobacilli, Nos. 73 and 74, which formed different proportions of lactic acid to succinic acid in (i) were each inoculated into a series of tubes containing the malic acid medium adjusted to pH values from 3·6 to 5·2 at intervals of 0·2 pH units. After incubation the acid end-products were determined as in (i). At pH 3·6, organism 73 produced lactic acid with a little succinic acid, while organism 74 formed lactic acid with only a trace of succinic acid. At pH 4·4, the maximum pH of cider-apple juices, organism 73 produced equal amounts of lactic and succinic acids, while organism 74 formed lactic acid with only a trace of succinic acid. At pH 5·2, organism 73 yielded mainly succinic acid with a trace of lactic acid, and organism 74 formed almost equal amounts of these acids.

Discussion.

The experiments described above show pH to be an important factor in the growth and metabolism of lactic acid bacteria. Experiment 1 showed that at the pH value of a full sharp juice (pH 3·1), the growth of the bacterium used was completely inhibited even during prolonged storage of the cider on the lees. The fact that both the natural Bramley juice and the Sweet Alford juice adjusted to a similar pH showed the same result, indicates that pH was the main factor controlling the growth of this organism. Suverkrop and Tchelistcheff (6) state that below pH 3·1 the speed of the malo-lactic fermentation in wines is decreased, sometimes taking up to 3 years to reach completion. It seems that a pH of 3·1 is likewise too low for the growth of the typical lactobacilli present in English ciders.

While a pH of about 3·4 permitted the growth of the test bacterium, growth was very slow until the ciders had reached dryness. At a pH of 4·0, a more rapid rate of growth of this organism enabled it to compete successfully with the yeast at S.G. 1·021. Autolysis of yeasts, with liberation of amino-acids, peptides and growth factors into the cider (see p. 174), may play a part in this respect, as Fornachon (7) has shown that autolysis occurs more quickly at the higher pH levels.

In view of the precautions taken to exclude contaminants, the large amount of acetic acid formed by the test organism is difficult to explain. Unlike the normal homofermentative lactic acid bacteria, the test organism produces catalase. It might, therefore, be expected to differ in other metabolic activities. A detailed investigation of this organism's metabolism of malic acid and fructose is now in progress.

Other strains of lactobacilli may compete even more successfully with the yeasts in juices at pH 4·0, particularly if the initial bacterial population of the juice is abnormally high. Experiment 2 throws some light on the early growth of bacteria in juices. The Gloucester sample of juice showed not only malo-lactic fermentation but also production of lactic acid from sugar. The Worcester juice was notable for the rapid and complete fermentation of malic to succinic acid. The lactic acid bacteria grew more slowly than the acetic acid bacteria,

and the numbers were small compared with the marked chemical change observed during the four days' incubation. The possibility that organisms other than lactobacilli bring about malo-succinic fermentation therefore cannot be ruled out.

Experiment 3 showed that lactobacilli isolated in pure culture from English ciders may convert malic acid to lactic and succinic acids, the proportion of the latter increasing with increasing pH. While lactic acid bacteria may be implicated in the pure malo-succinic change observed in Experiment 2 and in the work cited in the introduction, a lactobacillus capable of carrying out this change at a pH as low as 4·4 has not yet been isolated. It is therefore conceivable that organisms other than lactobacilli may be responsible for this change in ciders with pHs in this region.

Summary.

It is shown that the pH of a juice is an important factor in the growth and metabolism of lactobacilli. Malo-lactic fermentation is completely inhibited at the pH value of a full sharp juice ; it is delayed until dryness at the pH of a medium sharp juice, while at the pH level of sweet and bittersweet varieties, malo-lactic fermentation may occur very soon after pressing, particularly if the initial bacterial population is high.

Some lactobacilli ferment malic acid to a mixture of lactic and succinic acids, the proportion of the latter increasing with a rise in pH of the initial medium. Pure malo-succinic acid fermentations may occur in sweet or bittersweet juices, causing the pH to rise slightly while the titratable acidity remains constant.

Acknowledgments.

The authors wish to thank Mrs. J. M. Fielding, Mr. R. A. Coggins and Mr. J. H. Llewellyn for their technical assistance during this work.

REFERENCES.

(1) Carr, J. G. (1953). The lactic acid bacteria of cider : I. Some organisms responsible for the malo-lactic fermentation. *Ann. Rep. Long Ashton Res. Sta.*, 1952, 144.
(2) Steuart, D. W. (1949). The role of the heterofermentative lactobacilli in cyder-making. *J. Soc. chem. Ind., Lond.*, **68,** 250.
(3) Marshall, C. R. (1952). Some observations on the malo-lactic fermentation in cider. *J. Sci. Fd Agric.*, **3,** 91.
(4) Williams, A. H. (1950). Unpublished work.
(5) Phillips, J. D. (the late), Pollard, A. and Whiting, G. C. (1956). Organic acid metabolism in cider and perry fermentations : I. A preliminary study. *J. Sci. Fd Agric.*, **7,** 31.
(6) Suverkrop, B. and Tchelistcheff, A. (1949). Malo-lactic fermentation in California wines. *Wines and Vines*, **30,** 19.
(7) Fornachon, J. C. M. (1943). *Bacterial spoilage of fortified wines.* Australian Wine Board, Adelaide.

ACID CHANGES IN CIDER FERMENTATIONS: III. THE EFFECT OF SULPHITE TREATMENTS.

By F. W. BEECH, L. F. BURROUGHS and G. C. WHITING.

In previous years the addition of sulphur dioxide to a juice before fermentation has been found to prevent acidity changes and to give better flavoured ciders (1, and unpublished data). These effects were examined in more detail in the experiments described in this paper, where different amounts of sulphur dioxide were added either to the juice or to the dry cider and the influence of these additions on the flavour and acidity of the cider was investigated. Since the pH of the juice or cider greatly affects the growth of lactic acid bacteria, the experiment was made with both a low acid (Sweet Alford) and a high acid variety (Bramley's Seedling).

Experimental.

The same procedure was used for both Sweet Alford and Bramley's Seedling juices. Each juice was divided into three parts :—

A. Control, no SO_2 added.
B. 100 p.p.m. SO_2 added to juice.
C. 150 p.p.m. SO_2 ,, ,, ,,

No additions of yeast were made and the three juices were allowed to ferment to dryness, with adequate precautions against aerobic acetification.

Ciders A and B were each divided into two portions, one half being made up to 150 p.p.m. SO_2 and the other left untouched. Cider C, which originally had 150 p.p.m. SO_2 added to the juice, did not receive any additional SO_2.

This gave a total of five ciders, each of which was subdivided into three parts. Two parts of each were filtered clear (but not sterile) and stored at 5°C. (40°F.) and 15°C. (60°F.) respectively, while the remainder was left on its lees and stored at 15°C.

To avoid contamination with extraneous micro-organisms, sterile glass vessels were used for all fermentation and storage treatments. After storing for one month each cider was clarified by filtration, sweetened to S.G. 1.012, lightly carbonated, bottled and pasteurised.

Results.

1. *Low acid cider.* (Sweet Alford ; acidity 0·2% ; pH 3·9).

Acidity changes during fermentation and during storage of the ciders at 15°C. are shown in Table I. Ciders stored at 5°C. were almost identical in acid composition with the corresponding filtered ciders stored at 15°C. and are therefore omitted from the table. All ciders contained traces of succinic acid, of the order of 0·05% ; data for quinic, dihydroshikimic, chlorogenic and caffeic acids were also obtained but are not quoted here.

Acidity changes and flavour assessments of the ciders are summarized below.

A. Control : no SO_2 added to juice. During fermentation the titratable acidity (as % malic acid) increased from 0·20% in the juice to 0·48% in the dry cider. All the malic acid was converted to lactic acid and there was also

TABLE I.

SWEET ALFORD : ACIDITY CHANGES DURING FERMENTATION AND STORAGE.

Sample	Addition of SO_2 : to juice	Addition of SO_2 : to dry cider	Storage : filtered or on lees	Acidity as malic acid (g./100 ml.) Titratable	Acidity as malic acid (g./100 ml.) Total*	pH	Malic acid (g./100 ml.)	Lactic acid (g./100 ml.)	Volatile acidity as acetic acid (g./100 ml.)
Juice	—	—	—	0·20	0·38	3·93	0·27	nil	0·01
Cider A : at dryness ..	none.	—	—	0·48	0·68	3·78	nil	0·32	0·15
after storage ..	,,	none.	filtered.	0·47	0·68	3·89	nil	0·40	0·16
,, ,, ..	,,	,,	on lees.	0·50	0·70	3·90	nil	0·39	0·17
,, ,, ..	,,	150 p.p.m.	filtered.	0·49	0·69	3·87	nil	0·40	0·15
,, ,, ..	,,	,, ,,	on lees.	0·49	0·70	3·85	nil	0·40	0·17
Cider B : at dryness ..	100 p.p.m.	—	—	0·29	0·52	3·90	0·11	0·13	0·04
after storage ..	,,	none.	filtered.	0·29	0·50	3·89	0·11	0·16	0·04
,, ,, ..	,,	,,	on lees.	0·28	0·49	3·93	nil	0·26	0·08
,, ,, ..	,,	50 p.p.m.	filtered.	0·30	0·51	3·78	0·11	0·13	0·04
,, ,, ..	,,	,, ,,	on lees.	0·27	0·47	3·91	nil	0·25	0·05
Cider C : at dryness ..	150 p.p.m.	—	—	0·38	0·59	3·62	0·32	tr.	0·01
after storage ..	,,	none.	filtered.	0·38	0·59	3·66	0·32	tr.	0·01
,, ,, ..	,,	,,	on lees.	0·38	0·59	3·64	0·32	tr.	0·01

* Total acidity was determined by titration after removal of basic constituents of the sample by a cation exchange resin. It represents titratable plus combined acidity.

additional production of lactic acid and of acetic acid, presumably from sugars.

Since all acid changes were virtually complete in the dry ciders, little further change occurred during storage. All six ciders were acetic and unpleasant in flavour, especially those stored on the lees.

B. 100 *p.p.m.* SO_2 *added to the juice.* The titratable acidity increased only slightly during fermentation : from 0·22% in the juice to 0·29% in the dry cider. Approximately half the malic acid was converted to lactic acid and there was again a small additional production of lactic and acetic acids. A small amount of the " unfermentable " sugar xylose was present.

Only the two ciders stored on their lees showed any further acid changes. In these, malic acid was completely converted to lactic and there was some further production of lactic and acetic acids—probably from xylose which in the resulphited cider was greatly reduced and in the other had completely disappeared.

The flavour of the ciders in this group showed considerable variation. The best was the re-sulphited cider stored at 5°C. while that stored at 15°C. was only slightly inferior. The ciders stored without further addition of SO_2 were less attractive. Those stored on the lees were much inferior ; both were thin and lacking character and one was slightly tainted.

C. 150 *p.p.m.* SO_2 *added to juice.* The titratable acidity increased during fermentation from 0·22% in the juice to 0·38% in the dry cider. This increase was due to the production of an acid which is provisionally identified as malic. (In view of this unexpected result, other ciders from sulphited juices are being examined : this increase appears to occur widely in low acid juices). The dry cider contained only traces of lactic acid, so that lactic production either from malic acid or from sugars was strongly inhibited at this level of SO_2 ; a small amount of xylose was found.

No further change in acidity was found in any cider after storage ; xylose was still present. The flavours of the three ciders were similar and of high quality, fruity and full-bodied. They were much the best of all the ciders in this experiment.

2. *High acid cider.* (Bramley's Seedling ; acidity 0·95% ; pH 3·1).

In an exactly similar experiment with Bramley's Seedling juice, acid changes were completely inhibited by the low pH, even in the absence of SO_2. Small amounts of xylose were present throughout. The ciders were all very similar in flavour.

Discussion.

From these experiments with Sweet Alford and Bramley's Seedling juices, it is obvious that the pH of a juice is the main factor determining its susceptibility to bacterial acid changes. This conclusion, which is supported by the results in the preceding paper, has an important bearing in practical cider-making when a high proportion of culinary fruit has to be used. Under these circumstances malo-lactic fermentation is to be encouraged, and it is often necessary to leave ciders on their lees for considerable periods before malo-lactic bacteria develop. In the present experiment, the period of one month's storage on the lees was obviously insufficient for this to happen in the Bramley's Seedling ciders.

The reduction in acidity that may occur during fermentation or, more usually, during storage of medium and low acid ciders, is normally regarded as undesirable and may seriously affect the blending programme of a factory. The cider from the un-sulphited Sweet Alford juice is a good example of complete malo-lactic fermentation occurring before the cider was dry, but the resulting decrease in acidity was completely masked by the production of lactic and acetic acids from sugars. The addition of 150 p.p.m. SO_2 to the juice completely prevented these changes during fermentation and even during storage for one month on the lees.

When only 100 p.p.m. SO_2 was added to the juice there was a partial control of acid changes during fermentation, but conditions of storage were critical, and contact with the lees allowed acid changes to go to completion. At this intermediate level of SO_2, other juices of high pH may be even more susceptible to bacterial development, and it is therefore essential to clarify all medium- and low-acid ciders immediately after fermentation, and to store at a low temperature if acid changes are to be avoided. The same is advisable even where 150 p.p.m. SO_2 has been used, since some juices may be particularly susceptible to acid changes, either because of their chemical composition or of their bacterial flora.

A point of particular importance emphasized by these tests is that as much as 0·15% of acetic acid was formed during fermentation in the absence of SO_2 whereas only 0·01% was formed in the sulphited ciders (150 p.p.m.). Since the production of acetic acid in this way cannot be controlled by exclusion of air, this is a strong reason for sulphiting all low acid juices. Furthermore, the addition of 150 p.p.m. SO_2 to the Sweet Alford juice gave ciders of much better flavour than those from un-sulphited juice and there was no deterioration during storage. On the other hand, when only 100 p.p.m. SO_2 was added to the juice, storage at 15°C. had a bad effect on flavour, especially in the presence of lees, when there was loss of character sometimes accompanied by a taint. Although these flavour effects were due in part to the acid changes described above, other features of the flavour were also affected.

Summary.

(1) The effects of the addition of SO_2, and conditions of storage, on the acid changes and flavour of ciders have been investigated, using juices of low and high acidity.

(2) The addition of 150 p.p.m. SO_2 to the low acid juice completely prevented acid changes (due to lactic acid bacteria) during fermentation and storage of the dry cider.

The addition of 100 p.p.m. SO_2 to the juice partially inhibited acid changes during fermentation, but further changes occurred during storage, especially in the presence of lees.

In the absence of SO_2, acid changes were complete in the dry cider before storage.

(3) In the high acid juice, lactic acid bacteria did not develop, even in the absence of SO_2; no appreciable acid changes were observed.

(4) Addition of 150 p.p.m. SO_2 to the low acid juice gave ciders of good flavour, whereas the unsulphited ciders, especially if left on the lees, were tainted.

Acknowledgments.

The authors are indebted to Mr. R. A. Coggins for chromatographic determination of organic acids and to Mr. J. H. Llewellyn for the experimental treatment of the ciders.

REFERENCE.

(1) Beech, F. W. (1953). *Ann. Rep. Long Ashton Res. Sta.*, 1952, 125.

ACID CHANGES IN CIDER FERMENTATIONS: IV. YEAST AUTOLYSIS AND CIDER STABILITY.

By L. F. BURROUGHS.

Disorders due to lactic acid bacteria frequently occur in low acid ciders during the later stages of fermentation and during storage of the dry cider. These disorders are of three main types: (i) Ropiness or oiliness, the production of mucilaginous material; (ii) Malo-lactic fermentation, the decomposition of malic acid into lactic acid and carbon dioxide; (iii) Lactic acid production from sugar, sometimes accompanied by the formation of acetic acid. Combinations of these disorders may occur in the same cider and in some instances may be different manifestations of the same organism.

Past experience has indicated that these disorders are prevalent in ciders left in contact with their lees, and that such ciders, unless sterilised, may be subject to bacterial development after bottling. An experiment was therefore designed to investigate the relationship between the extent of yeast autolysis in a cider and the development of lactic acid bacteria after bottling under non-sterile conditions.

Experimental.

The variety Morgan Sweet was chosen for this work since it is well known for the rapid fermentation of its juices, indicating the growth of a relatively large crop of yeast, and for the susceptibility of its ciders to bacterial attack.

Three ciders were prepared as described below :—

Cider A. Normal fermentation : fermented to dryness (4 weeks) and racked from the lees.

Cider B. Autolysed : fermented to dryness and stored on the lees.

Cider C. Controlled fermentation : the cider was centrifuged at a fast rate of flow after six days; it then fermented slowly to dryness (12 weeks) and was racked from the lees.

The dry ciders were stored in 18 gallon casks before bottling. Since Cider C had fermented more slowly than Ciders A and B, its period of storage (14 weeks) was necessarily less than that of the other two ciders (24 weeks).

After storage, each cider was sweetened to S.G. 1,020, filtered clear (but not sterile) and filled aseptically into sterile bottles. Twelve bottles of each cider were stored at each of three temperatures : (i) in the cellar, averaging 12°C. (54°F.); (ii) at 15°C. (60°F.); (iii) at 25°C. (77°F.).

Results.

The original juice had the following analysis : Specific gravity 1,045; Titratable acidity 0·26% (as malic acid); pH 3·93. The titratable acidity increased slightly during fermentation to 0·28%; pH remained unchanged.

The soluble nitrogen contents of the juice and ciders, determined by micro-Kjeldahl digestion of centrifuged samples, are given below.

Juice	.. ..	..	13·5 mg.N/100 ml.
Centrifuged cider (S.G. 1,032)	.. ..	2·3	,, ,,
Ciders at time of bottling :—			
A — Normal fermentation	.. ..	2·0	,, ,,
B — Autolysed cider	.. ..	3·7	,, ,,
C — Controlled fermentation	..	1·1	,, ,,

The amino acids, which are the chief components of the soluble nitrogen in juices and ciders, were examined chromatographically in these samples, and a useful indication was obtained of the extent of yeast autolysis in the ciders. Details of this work will be published elsewhere, but in this paper use is made of the chromatographic observations in interpreting the soluble nitrogen data.

The outstanding feature of these results is the low soluble nitrogen content of the centrifuged cider, showing that most of the nitrogen of the original juice was utilized for yeast growth during the first six days for a loss of only 13 degrees of specific gravity. In the undisturbed fermentations of Ciders A and B, it can be assumed that the uptake of nitrogen followed a similar course and yeast growth during the rest of the fermentation was therefore small. By analogy with other ciders (1) it is probable that while the uptake of juice nitrogen continued slowly, at the same time nitrogen was returned to the cider by autolysing yeasts and that as the cider approached dryness the soluble nitrogen content actually increased. This effect of autolysis was clearly shown in Cider B (left on its lees) where the nitrogen content rose to 3·7 mg./100 ml. due to the excretion of amino acids, particularly valine, leucine, arginine and lysine. Tyrosine and phenylalanine, which are not normally found in juices and ciders, were also present. Cider A (2·0 mg.N/100 ml.), which had been racked from the lees at dryness, had a similar amino acid composition but the increases were less marked and tyrosine and phenylalanine were not detected. In contrast, the soluble nitrogen content of Cider C was very low (1·1 mg./100 ml.) due to the more complete utilization of the small amount of nitrogen left in the cider after centrifuging. Since only a small yeast crop was able to develop, the effects of yeast autolysis were negligible in spite of the protracted fermentation. This cider contained only traces of amino acids.

In addition to amino acids, the products of yeast autolysis are known to include other substances (vitamins and growth factors) essential for the growth of bacteria. These, together with the amino acids simultaneously liberated, would render an autolysed cider more vulnerable to bacterial attack (2, 3).

Changes during Storage.

The bottled ciders were examined after three months storage at three different temperatures. The appearance of the ciders in bottle was as follows :
Cider A : all brilliant but considerable deposits at all temperatures.
Cider B : heavy deposits at all temperatures; ciders at 15°C. and 25°C. were hazy.
Cider C : all brilliant and only traces of deposit at all temperatures.

Deposits from the ciders stored at 25°C. were examined microscopically :—
Cider A contained moderate numbers of yeast cells and rod-shaped bacteria (lactobacilli).

Cider B was very heavily populated with lactobacilli and possibly some cocci ; very few yeast cells were present.

Cider C contained a few yeasts and lactobacilli.

The ciders were analysed to determine changes that occurred during storage in bottle. Only small decreases in specific gravity were observed : the chief differences were in titratable acidity (Table I), acid production being favoured by the higher storage temperatures.

TABLE I.

INCREASES IN TITRATABLE ACIDITY OF CIDERS DURING STORAGE AT DIFFERENT TEMPERATURES, (AVERAGES OF TRIPLICATE BOTTLES).

Cider	Temperature of Storage		
	Cellar	15°C.	25°C.
A. Normal Fermentation	0·01	0·02	0·05
B. Autolysed Cider	0·01	0·26	0·51
C. Controlled Fermentation	0·00	0·00	– 0·02

The nature of the acids present in the ciders stored at 25°C. was examined chromatographically. It was found that all ciders had undergone a malo-lactic fermentation and that in addition there was a considerable production of lactic and acetic acids, presumably from sugar, in cider B and to a much less extent in Ciders A and C. The volatile acidities (expressed as acetic acid) of the ciders stored at 25°C. were : A, 0·12% ; B, 0·34% ; C, 0·10%.

Comments on the flavour of the ciders are summarized below :—

Cider A : although the cider stored at cellar temperature was clean and of average quality, those stored at higher temperatures were tainted and unpleasant.

Cider B : all samples were unclean ; they were yeasty at cellar temperature, acetic at 15°C. and badly acetic at 25°C.

Cider C : all samples were clean in aroma and flavour.

From the practical point of view, Ciders A and B would be regarded as having an unsatisfactory storage life.

The incidence of bacterial spoilage in these low acid ciders was directly related to the extent to which yeast autolysis had been allowed to occur during fermentation and cask-storage. Cider A represents the normal practice of commercial cider-making, namely fermentation to dryness and racking from the lees, while the practice of leaving cider on its lees (Cider B) is not uncommon in farm cider-making. The amount of autolysis products liberated depends on the amount of yeast present and the duration of its contact with the cider. It is probable, therefore, that some degree of autolysis occurs in all ciders and that when this becomes excessive it may encourage the growth of micro-organisms during storage and may adversely affect the flavour of the cider.

The main effects are acidity changes and loss of fruity character, often associated with acetification and the development of taints. Bacterial fermentation in the bottle is only likely to arise when a badly affected cider is used in a blend of low acidity (high pH) and conditions of bottling allow organisms from the cider, or other source, to enter the finished product. This combination of circumstances would be uncommon in modern commercial practice : it was deliberately chosen in this experiment to demonstrate the inadvisability of leaving cider on its lees.

The procedure of centrifuging during the early stages of fermentation (Cider C) would not be applicable to all ciders, since rates of fermentation would then be inconveniently slow. Some removal of yeast from the more vigorously fermenting ciders by centrifuging or racking at an early stage would, however, prevent excessive autolysis and consequent risk of bacterial fermentation. This has already been advocated in an earlier article (1).

Although the development of lactic acid bacteria in a low acid cider can be effectively controlled by adding sulphur dioxide to the juice (see p. 169) this procedure may not always be possible. Under these circumstances the necessity of minimizing yeast autolysis is especially important.

Summary.

(1) Ciders of low, medium and high nitrogen content were produced from the same juice (Sweet Alford) by controlling the extent of yeast autolysis.

(2) Development of lactic acid bacteria in the ciders after bottling was directly related to the extent of yeast autolysis and resulted in deposit formation, increases in acidity and deterioration of flavour.

Acknowledgments.

The author is indebted to Mr. J. G. Carr for microscopic observations and to Dr. G. C. Whiting for chromatographic examination of organic acids. He also wishes to thank Miss Shirley J. Locke and Mr. B. L. Davies for assistance with analytical work, and Mr. J. H. Llewellyn for the experimental treatment of the ciders.

REFERENCES.

(1) Burroughs, L. F. and Pollard, A. (1954). *Ann. Rep. Long Ashton Res. Sta.*, 1953, 184.
(2) Vaughn, Reese H. (1955). *Advances in Food Research*, **6**, 85.
(3) Fornachon, J. C. M. (1943). *Bacterial spoilage of fortified wines.* (Austral. Wine Board Adelaide), 79.

A COMPARISON OF SOME VARIETIES OF VEGETABLES PRESERVED BY CANNING AND BY FREEZING: PROGRESS REPORT IV.

By ALICE CRANG and MARGARET STURDY.

An assessment of the preserving qualities of four varieties of broad beans, tested for three of the years between 1950 and 1955, is given in this report. The effects of maturity and method of cutting on the preserving qualities, and the wastage in preparing beans and peas have been determined.

Experimental.

Vegetables Used. The vegetables were all grown at Long Ashton and picked when slightly immature and also when at their prime, most varieties being picked on three or more days during the season. They were preserved by deep freezing or canning as soon as possible after picking.

Preparation. The broad beans and peas were shelled in the usual way. The ends of dwarf and runner beans were cut off and the strings removed where necessary. The smaller beans were preserved whole and the larger ones sliced thinly or cut diagonally into slices about an inch wide.

Preserving and Judging Methods. The methods of canning, freezing and of judging the preserved products were the same as those used previously (1).

Results.

The mean marks awarded to the broad bean varieties tested for three years are given in Table I. Of these varieties, only Triple White gave an acceptable product when preserved by canning, and even this variety was rated considerably higher for colour and flavour when frozen. All the varieties were considered suitable for freezing.

TABLE I.

BROAD BEANS.

Variety	Process	Colour		Texture		Flavour	
		Mean	Range	Mean	Range	Mean	Range
Harlington White ..	Frozen	8·0	(7·6–8·3)	7·9	(7·1–8·5)	7·9	(7·4–8·1)
Taylor's Windsor White..	„	8·1	(7·5–8·6)	8·0	(7·5–8·2)	7·8	(7·4–8·3)
Sussex Wonder	„	8·6	(8·0–9·2)	7·4	(6·3–8·2)	7·8	(7·5–8·1)
Triple White	„	8·7	(8·4–9·3)	6·8	(5·5–8·0)	7·6	(6·9–8·0)
„ „	Canned	6·4	(5·8–7·3)	7·5	(7·2–8·0)	6·6	(6·0–7·1)

The differences in quality of the vegetables preserved at different stages of maturity and the variations due to different methods of cutting are shown in Table II.

TABLE II.

THE EFFECTS OF MATURITY AND METHOD OF CUTTING ON QUALITY.

	Colour		Texture		Flavour	
	Canned	Frozen	Canned	Frozen	Canned	Frozen
Beans, Broad : (Mean of 5 varieties)						
Slightly immature	—	8·1	—	8·0	—	7·8
Mature	—	7·9	—	6·8	—	8·3
Beans, Dwarf, whole : (Mean of 7 varieties)						
Slightly immature	—	7·5	—	8·2	—	7·3
Mature	6·9	7·3	7·7	7·9	6·8	7·1
Beans, Dwarf : (Mean of 4 varieties)						
Slightly immature, whole	—	7·3	—	7·4	—	6·7
Mature, sliced	6·9	7·9	7·7	7·5	6·8	7·2
Beans, runner : (Mean of 2 varieties)						
Slightly immature, sliced thinly	—	9·6	—	8·4	—	7·3
Mature, sliced thinly	7·3	9·3	8·7	8·3	7·4	7·5
Mature, slices 1″ across	7·0	8·7	8·8	8·4	7·5	7·7
Peas : (Mean of 6 varieties)						
Slightly immature	6·3	7·7	7·3	8·3	5·6	7·4
Mature	6·8	8·9	7·3	7·6	6·5	7·8

TABLE III.

WASTE ON PREPARATION.
(Percentage of Total Weight).

	Mean	Range
Beans, Broad : (Mean of five varieties)		
Slightly immature	73	57 – 82
Mature	64	45 – 75
Beans, Dwarf and Runner :		
Stringless (Mean of five varieties)		
Slightly immature	9	4 – 13
Mature	8	6 – 9
Strings removed (Mean of three varieties)		
Slightly immature	25	11 – 34
Mature	24	21 – 28
Peas : (Mean of six varieties)		
Slightly immature	70	58 – 82
Mature	62	51 – 74

From these results it will be seen that the texture of the slightly immature broad beans was preferred to that of the mature ones, but the latter were a little better flavoured.

There was a slight preference for the immature dwarf beans over the more mature ones when both could be preserved whole, but not when the mature pods were of a size that needed slicing.

The thinly sliced runner beans were preferred to the coarser cut ones for colour, but not for flavour ; the differences in the marks awarded for texture were negligible.

The mature peas were rated higher for both colour and flavour when canned or frozen than those picked at a younger stage.

All the canned beans and peas were rated considerably poorer in colour and a little poorer in flavour than the corresponding frozen samples.

The amount of wastage in the preparation of the beans and peas is given in Table III.

Discussion.

Beans, Broad.

The varieties Harlington White, Taylor's Windsor White and Sussex Wonder have given very similar results in the three years tested ; all have proved satisfactory for freezing, but the canned beans were quite unacceptable when tested in 1952 (2) and further canning of these varieties has not been attempted. The variety Triple White has proved satisfactory for freezing, and is the only variety of broad bean tested so far in these trials that has given an acceptable product when canned.

The results obtained were similar to those found previously (1) in that the immature beans were preferred for texture, but there was no appreciable difference in colour and flavour due to maturity. The wastage in preparation was about 9% less in the mature beans than in the immature.

Beans, Dwarf and Runner.

Comments on individual varieties are not given in this report, as those included in these trials have been tested for only one or two seasons.

The differences in quality due to maturity have been small, and less than those due to the method of preparation. The thinly sliced beans have given a product with a better colour, but weaker flavour, than those either cut more coarsely or left whole. These results are in agreement with those found for the varieties tested in 1954 (1).

There was only a slightly greater percentage loss in preparing the immature than the mature beans, but since there was no material improvement in quality, it would obviously be of advantage to preserve the fully grown beans. The waste that resulted from stringing was about 16%. Five varieties were, in error, preserved as " stringless " whereas the strings were objectionable in the preserved mature beans : these have not been included in the results given.

Peas.

The mature peas were preferred both for colour and flavour to those preserved at an earlier stage, and as there was 8% less waste in podding, there seems no advantage in preserving the younger ones.

Summary.

The assessment of colour, texture and flavour of four varieties of broad beans tested for three years since 1950 is given. Only one of these, Triple White, was found suitable for canning, though all were satisfactory for freezing.

The results of comparing peas and beans preserved at different stages of maturity were similar to those obtained in the previous year (1) in that a slight degree of immaturity improved the texture of broad beans, but weakened the flavour of broad and runner beans and of peas; losses in preparation were greater for immature samples than for mature ones.

Thinly sliced runner beans were again rated higher for colour but lower in flavour than those sliced diagonally into pieces about an inch thick.

Frozen peas and beans were all rated considerably higher than canned ones for colour, and higher for flavour.

The average wastage in preparation of slightly immature peas and beans and mature ones was respectively :—broad beans, 73 and 64%; stringless dwarf and runner beans, 9 and 8%; other dwarf and runner beans, 25 and 24%; peas 70 and 62%.

REFERENCES.

(1) Crang, A. and Sturdy, M. (1955). *Ann. Rep. Long Ashton Res. Sta.*, 1954, 187–191.
(2) Crang, A. and Sturdy, M. (1953). *Ibid.*, 1952, 185–191.

A COMPARISON OF SOME VARIETIES OF FRUIT PRESERVED BY BOTTLING AND BY FREEZING: PROGRESS REPORT VI.

By ALICE CRANG and MARGARET STURDY.

This report gives an assessment of the qualities of fourteen varieties of raspberry, nine varieties of strawberry and eight varieties of pear examined in 1955. The raspberries and strawberries were compared when fresh and again after bottling and after deep-freezing; the pears were judged when fresh and after bottling only: previous trials (1) had shown them to be unsuited to freezing.

All the raspberries were grown at the National Agricultural Advisory Service Sub-Centre, Starcross; of the strawberries, Cambridge Rival and Cambridge Vigour were grown at Starcross, Majestic (new variety) at Conway, Royal Sovereign at Long Ashton and the remainder at Cheddar; the pears were grown at Long Ashton.

Preserving and Judging Methods.

Fruit from Starcross was transported and held overnight in insulated boxes containing solid carbon dioxide: strawberries from other sources were held in the same containers overnight. The fruit was preserved on the following day after being judged in the fresh state, with and without the addition of sugar. The methods of preservation were the same as those used previously (2) except that instead of a liquid red colour, Ponceau 2R (2·5 g. per gallon syrup) was used in the bottled strawberries. No artificial colour was used with the raspberries or with the frozen fruit.

The pears were ripened at room temperature, and those varieties ripening together were compared for fresh fruit quality. For bottling, 13 fl. oz. of 30° Brix sugar syrup were used with 19 oz. of peeled halved pears.

For the first time in these trials the effect of storage temperature on the bottled fruits was noted. Two bottles of each raspberry and strawberry variety were prepared, one stored at room temperature (mean 69°F. during the summer months) and the other at a mean temperature of 38°F. throughout.

The raspberries were judged after 4 months; strawberries after 4½ months; and the pears after 1 to 2 months. The figures given (Tables I-III) are the mean marks allocated by 12 to 14 tasters, using the method of marking previously described (1).

Raspberries.

Table I gives the marks awarded for the fresh, frozen and bottled raspberries. Although some of the fruit was picked after a heavy rainstorm, the quality was generally better than in 1954 and 1953, for which mean values are quoted. (The varieties tested in these two years included 13 and 9, respectively, of those examined in 1955). Much of the improvement in quality was undoubtedly due to the generally warm, sunny weather during the ripening season (maximum temperature 6°F. above average) but cooling the fruit with solid carbon dioxide during transport may have been contributory.

TABLE I.

RASPBERRIES.

Variety	Colour				Texture				Flavour				
	Fresh	Frozen	Bottled W.*	Bottled C.*	Fresh	Frozen	Bottled W.*	Bottled C.*	Fresh φ N.S.	Fresh φ S.	Frozen	Bottled W.*	Bottled C.*
Early Red	7·6	8·3	7·5	8·6	7·9	6·7	7·3	7·6	8·2	8·6	7·1	7·9	7·9
Lloyd George	6·6	8·3	6·8	8·6	8·3	6·9	7·2	7·6	8·1	8·7	7·1	7·1	7·6
Malling Enterprise	8·3	7·9	5·8	7·5	8·4	6·9	7·4	7·9	7·0	7·5	6·9	6·5	7·3
Malling Exploit	7·7	7·0	5·7	6·8	8·2	8·1	7·1	7·5	6·0	6·4	7·5	6·8	7·2
Malling Jewel	8·8	7·2	5·3	7·2	8·4	7·6	7·2	7·9	6·6	7·4	6·9	6·6	7·3
Malling Landmark	6·9	6·8	5·3	7·4	7·5	6·6	6·8	7·2	7·0	7·7	7·0	6·9	7·3
Malling Promise	7·9	8·1	7·0	7·5	8·2	7·6	7·2	7·9	6·9	7·3	6·9	6·6	8·1
Malling V	8·4	7·7	6·5	8·2	8·4	7·7	7·1	7·8	7·0	7·2	7·2	7·3	8·0
Malling W	8·2	7·6	6·9	8·2	8·1	6·6	6·8	8·0	6·7	7·2	7·4	7·6	7·7
Malling Y	7·5	7·9	6·0	8·1	7·6	7·6	7·0	7·5	6·4	6·7	6·6	7·0	7·8
Malling Z	7·5	7·3	5·5	6·9	7·5	6·9	6·5	7·0	6·5	6·9	7·1	6·8	7·5
Milton	6·9	7·5	5·6	6·4	7·3	8·1	7·0	7·0	6·6	7·3	7·7	6·4	7·4
Newburgh	7·5	7·9	6·5	8·1	7·7	6·3	6·9	7·5	6·2	6·6	6·8	7·0	7·7
Preussen	7·2	7·3	5·8	6·6	7·2	6·0	7·2	7·2	5·5	5·7	6·4	6·4	7·3
Mean of 14 varieties 1955	7·6	7·6	6·2	7·6	7·9	7·1	7·1	7·5	6·8	7·2	7·0	6·9	7·6
Mean of 15 varieties 1954	7·5	7·3	6·1	—	7·6	7·2	6·8	—	6·0	—	6·9	6·9	—
Mean of 10 varieties 1953	7·4	7·3	5·6	—	7·5	6·9	6·2	—	6·3	—	6·4	6·3	—

*W.—Stored at approx. 69°F. φFresh N.S.—Tasted without sugar.
*C. —Stored at approx. 38°F. φFresh S. —Tasted with added sugar.

All the varieties were marked appreciably higher for flavour when sugar was added to the fresh fruit.

Preserving Methods.

Bottled fruit stored in the cold retained its colour so well that its average rating was as high as that of the fresh and frozen fruit, whilst its flavour was on the average judged to be better than that of fresh and frozen fruit. As in previous years bottled fruit stored at room temperature was of poor colour ; it was also inferior in texture and flavour to that stored in the cold.

Varieties.

Early Red. One of the most satisfactory varieties both fresh and preserved, combining good appearance and flavour.

Lloyd George. Rather too dark in colour when fresh ; this was an advantage when preserved. Flavour one of the best of the series.

Malling Enterprise. Very good appearance and fairly good flavour when fresh but not outstanding when preserved.

Malling Exploit. Too pale as bottled fruit, flavour throughout weak ; retained its shape well in processing.

Malling Jewel. Fresh fruit had very good appearance and fair flavour ; colour and texture not so good when preserved.

Malling Landmark. Too pale in colour and the drupelets easily separated on processing ; flavour fairly good.

Malling Promise. Appearance good throughout ; fair to good flavour.

Malling V. Very attractive fruit when fresh, with moderate flavour. Preserved better than many of the varieties.

Malling W. Similar in quality to the previous variety, but texture of the frozen fruit rather poorer.

Malling Y. Fairly good in appearance but of only fair flavour when fresh or frozen ; better when bottled.

Malling Z. Not outstanding in any way.

Milton. Disappointing when fresh or bottled ; rather better when frozen.

Newburgh. Flavour of fresh and frozen fruit below average ; cold-stored bottled fruit of good colour and flavour.

Preussen. Poorest of the varieties for flavour, and of only moderate appearance.

Strawberries.

The quality assessment for the fresh and preserved strawberries is given in Table II. The flavour of the fresh fruit was considered to be better than in 1954 and the addition of sugar further improved it. The mean values given for 1954 include six of the varieties judged in 1955.

Preserving Methods.

Strawberries require artificial colouring if they are to remain attractive after bottling. Previously (2) frozen fruit had proved greatly superior to bottled fruit in colour but, although in the present trials bottled fruit stored at room temperature was poorer in colour than frozen fruit, that kept in the cold was better. Bottled fruit stored in the cold was also of better flavour than that stored at room temperature ; both were rated higher than the frozen fruit.

TABLE II.

STRAWBERRIES.

Variety	Colour				Texture				Flavour				
	Fresh	Frozen	Bottled W.*	Bottled C.*	Fresh	Frozen	Bottled W.*	Bottled C.*	Fresh N.S.ϕ	Fresh S.ϕ	Frozen	Bottled W.*	Bottled C.*
Auchincruive Climax ..	8·6	6·9	6·3	7·9	8·3	7·2	7·3	8·2	7·7	8·2	6·4	7·2	7·6
Axbridge Early	7·0	7·7	7·0	7·3	7·1	7·5	7·0	7·0	6·9	7·5	6·4	7·0	7·2
Cambridge Favourite ..	8·1	7·7	7·6	8·1	8·3	7·5	8·2	7·9	7·6	8·2	6·4	7·7	7·0
Cambridge Prizewinner ..	9·3	7·3	7·0	7·6	7·7	7·2	7·0	7·3	6·7	7·1	6·8	6·1	7·8
Cambridge Rival	6·5	5·9	—†	7·9	7·6	6·7	—†	8·2	7·3	7·2	5·8	—†	7·3
Cambridge Vigour	7·9	7·5	6·7	7·9	8·6	7·8	8·0	8·3	7·4	7·7	6·3	7·2	7·7
Huxley	6·6	5·9	6·5	8·1	7·7	7·0	7·5	8·1	7·3	7·7	6·5	7·3	7·9
Majestic	9·5	6·6	—‡	8·2	9·1	6·8	—‡	7·4	7·4	7·9	6·2	—‡	6·3
Royal Sovereign	8·2	7·6	6·1	7·2	8·9	7·4	7·6	7·8	8·2	8·9	6·8	7·6	7·9
Mean of 9 varieties 1955 ..	8·0	7·0	6·7	7·8	8·1	7·2	7·5	7·8	7·4	7·8	6·4	7·2	7·4
Mean of 9 varieties 1954 ..	7·6	7·1	—	—	7·9	6·9	6·5	—	6·9	—	6·1	6·0	—

* W.—Stored at approx. 69°F.
* C.—Stored at approx. 38°F.
† Attacked by mould *Byssochlamys fulva*.
ϕ N.S.—Tasted without sugar.
ϕ S. —Tasted with sugar.
‡ Insufficient sample for all tests.

Varieties.

Auchincruive Climax. Very satisfactory when fresh, and bottled well; frozen fruit not rated as highly as previously.

Axbridge Early. Moderate quality throughout.

Cambridge Favourite. Satisfactory in appearance when fresh and preserved; flavour good except in frozen fruit, in which it was rather weak.

Cambridge Prizewinner. Rated very highly for fresh fruit colour, but flavour only fair; moderately good preserving qualities.

Cambridge Rival. Unattractive when fresh and frozen, but dark colour an asset in bottling; flavour fairly good when fresh and bottled, but poor when frozen.

Cambridge Vigour. Good colour and texture, and fairly good flavour.

Huxley. Better as artificially coloured bottled fruit than when fresh or frozen; flavour moderately good.

Majestic. Fresh fruit of exceptionally good colour and texture and of moderate flavour; bottled fruit poor in flavour. This new variety is worth further trial.

Royal Sovereign. The best flavoured fruit when fresh, and flavour above average when preserved; appearance this year good when fresh or frozen, but below average when bottled.

Pears.

The colour, texture and flavour ratings of the six varieties of dessert pears judged as fresh fruit, and of the eight included in the bottling trials, are given in Table III. All the varieties bottled satisfactorily.

Varieties.

Beurré Bedford. Only fair quality when fresh, but rated highly when bottled.

Beurré Hardy. Preferred to the previous variety when fresh; its appearance when bottled was good and flavour fairly good.

TABLE III.

PEARS.

Variety	Colour		Texture		Flavour	
	Fresh	Bottled	Fresh	Bottled	Fresh	Bottled
Beurré Bedford ..	7·2	8·6	7·8	8·3	6·6	8·7
Beurré Hardy ..	7·8	7·6	7·7	8·3	8·2	7·2
Bristol Cross ..	—	8·8	—	8·5	—	7·8
Comice	—	8·8	—	7·6	—	7·1
Conference ..	7·2	7·8	7·5	8·1	7·9	7·8
Durondeau ..	8·3	7·9	8·3	7·8	7·5	7·1
Gorham	8·5	8·8	8·9	8·4	8·5	8·2
Louise Bonne ..	7·9	7·0	7·6	8·4	7·2	8·2
Mean	—	8·2	—	8·2	—	7·8

Bristol Cross. Bottled well ; appearance very good and flavour good.

Comice. Very good in colour when bottled, but its flavour was not outstandingly good.

Conference. Appearance good when bottled ; flavour of fresh and bottled fruit good.

Durondeau. Good colour and flavour when fresh ; lost a little in flavour when bottled.

Gorham. One of the best varieties both as fresh and bottled fruit.

Louise Bonne. Rated good in appearance and fairly good for flavour when fresh ; on bottling lost a little in colour but improved in texture and flavour.

Summary.

Fourteen varieties of raspberry and nine varieties of strawberry were assessed during 1955 for their fresh fruit, bottling and freezing qualities. Colour and flavour were so well retained in bottled fruit stored at 38°F. that it was rated higher than frozen fruit : bottled fruit stored at room temperature was much poorer in appearance.

Eight varieties of pear were included in the bottling trials.

Acknowledgments.

We are indebted to Mr. F. A. Roach, Mr. W. C. Ibbett and Mr. E. N. Powell for obtaining the raspberries and strawberries, and to members of the Station staff for their help in preserving and judging the fruit.

REFERENCES.

(1) Crang, A., Kendall, L. and Sturdy, M. (1951). *Ann. Rep. Long Ashton Res. Sta.*, 1950, 197–207.

(2) Crang, A. and Sturdy, M. (1955). *Ann. Rep. Long Ashton Res. Sta.*, 1954, 192–198.

DOMESTIC FREEZING OF PORK AND PORK PRODUCTS.

By MARGARET STURDY and ALICE CRANG.

Many rural householders still fatten pigs for home consumption : much of the pork is cured and used later as bacon or ham, but as a proportion is unsuitable for curing there is a demand for alternative preserving methods. Deep-freezing is being increasingly used on farms and this would seem an obvious method to recommend.

Loss of quality in frozen foods is frequently due to inadequate wrapping which allows evaporation to occur. Mackey, Oliver and Fang (1) compared the shrinkage during the freezing of joints wrapped in different materials and found that, despite a gradual loss of flavour in the fat, frozen pork, when properly wrapped and stored at 0°F., retained its quality well for approximately one year. Noble and Hardy (2) found that the loss of moisture and fat during the storage, thawing and roasting of frozen pork was slightly less than that of similar fresh joints during roasting. Tressler and Evers (3a) state that pork held for not more than 48 hours at 32°F. before freezing will remain in good condition at 0°F. for approximately a year ; but if pork is held in a cooler for 8 or 10 days prior to freezing, the fat will turn rancid within a few months' storage at 0°F. Inadequate wrapping of food prior to freezing may also cause rancidity of the fat. In the condition known as " freezer burn " the fats immediately beneath the desiccated areas are oxidised and may be rancid ; the proteins are irreversibly dehydrated and consequently the tissues are dry and tough (3b).

The effect of salt on the onset of rancidity in frozen pork products has been noted by a number of workers. Wiesman and Ziemba (4) found that the omission of salt from pork sausages prevented discoloration of the lean and rancidity of the fat over a 6 months' storage period, whereas, in those containing salt storage under vacuum or in an inert atmosphere, with or without anti-oxidants, was ineffective. Chang and Watts (5), using a filter paper technique, found that sodium chloride, either dry or in concentrated solution, accelerated oxidation of fat. Others have recommended omitting salt from sausages prior to freezing and have yet included salt in meat loaf containing minced pork and have also recommended the freezing of cured meat (3c).

The following trials were carried out partly to demonstrate to students the right and wrong ways of freezing pork products and also to test the equipment and materials available in this country for deep-freezing.

Experimental.

During the period 1953 55 tests were made with a domestic deep-freeze unit to determine the best methods of packaging, freezing and cooking pork and pork products with the materials available to the housewife. Freezing temperatures were in the range —14° to —8°F. ; storage temperatures were 0°F. or lower. Fresh loins and chops, sausage meat and pickled pork were chosen as representative of the products most likely to be preserved in the home : they were stored over periods of four to twelve months.

Wrapping Materials.

Fruit or fruit pulp was first used to determine the moisture- and vapour-proof qualities of different materials and containers. The containers were filled with fruit or fruit pulp of known ascorbic acid content, sealed, weighed and placed in the freezer. After various intervals of storage, the containers were thawed to room temperature, and re-weighed after the removal of condensation. The ascorbic acid content of the fruit was again determined after 6 months' storage. The two most promising materials, polythene (polyethylene) and Cellophane grade MSAT 300 30, were then tested against greaseproof paper of household quality in a series of trials with pork chops. Seams of polythene and Cellophane packages were heat-sealed with a flat iron, a sheet of paper being used to prevent the polythene from sticking to the iron ; Scotch tape was used to secure the paper seams. All packages were wrapped in brown paper for protection during storage in the freezer. Aluminium foil and polythene were used in further tests with sausage meat.

Storage, Thawing and Cooking Tests.

Loins of Pork. Joints of approximately $1\frac{3}{4}$ lb. were cut from the loin of a Saddle-back pig killed 48 hours previously. The joints were numbered consecutively and wrapped in Cellophane and covered with brown paper before freezing. Two consecutive joints were removed at intervals of 6, 10 and 12 months : one of each pair was left wrapped to thaw at room temperature for 18 hours while the second wrapped joint was placed in a cold store (32–40°F.) for 24 hours. Weights of all joints were noted before freezing ; on removal from the freezer ; when thawed and drained and when cooked ; and the weight of dripping in the pan was noted. All the joints were cooked at 450°F. for 70 or 80 minutes. Contrary to normal practice the baking tins were uncovered and no cooking fat was added : this allowed the maximum losses on cooking to be recorded. The average temperature of joints thawed at room temperature was 56°F., and in the cold store 38°F., the latter requiring an extra 10 min. cooking time.

Pork Chops. Twelve lean chops were cut from a Berkshire hog five days after killing. Three adjacent chops were taken from the loin, weighed and wrapped either in (a) polythene-lined paper bag, heat-sealed, (b) Cellophane bag grade MSAT 300 30, heat-sealed or (c) grease-proof paper sealed with Scotch tape ; those wrapped by methods (b) or (c) were covered with brown paper for further protection in the freezer. Adjacent chops were removed at periods of 4, 10 and 12 months, thawed at room temperature for two hours, drained, grilled and compared with grilled fresh chops. Losses in weight were noted at each stage.

Pairs of chops were also sealed in polythene bags and greaseproof paper and cooked and tasted after 4 to 5 months' storage in the freezer.

Sausages. It was felt that many people might want to freeze sausages, but might not want to go to the trouble (or expense) of filling them into casings for home storage. Trials were therefore made using egg as a binding material, to prevent excessive losses on cooking the skinless sausages.

Sausage meat was prepared in the proportions :—

9 lb. lean pork	3 lb. moistened breadcrumbs
3 lb. pork fat	3 oz. salt $1\frac{3}{4}$ oz. spices.

A further batch was made with the addition of three beaten eggs, half added to the mixture and the remainder used for coating the sausages after they had been moulded into shape. Four sausages from each batch were weighed before and after cooking for 20 minutes in an oven at 300°F.

The Effect of Salt on Rancidity.

(1) *Sausage meat.* A shoulder of pork, received within 48 hours of the animal being slaughtered, was used in preparing sausage meat from the following recipe :—

8 lb. lean meat
4 lb. fat
3 lb. moistened breadcrumbs
4 oz. salt
Spices { ¾ oz. ground white pepper
{ ½ oz. each ground mace, nutmeg and coriander.

The sausage meat was divided into four equal portions : one unseasoned, one with salt, one with spice, one with salt and spice.

The sausage meat from each of these treatments was weighed into 4 oz. portions, wrapped in aluminium foil, with the edges carefully turned over to exclude air, and frozen. Two portions of each sample were tested after 3, 6 and 9 months, and the remaining samples after 19 months. They were removed from the freezer, weighed and thawed at room temperature for two hours, the foil being unwrapped only from the top of the sample, which was then placed on a baking sheet in an oven at 500°F. and baked for 30 minutes until brown on top.

A second series of sausages was prepared from the recipe containing egg ; these were moulded into shape, packed tightly in polythene bags, and sealed.

Pickled Pork and Chap. The forehock and chap (pig's jaw) were pickled for six days in a brine containing 20% salt and spices. The forehock was removed from the brine, boned, rolled and simmered for 2 hours ; it was then placed in a mould and weighted down whilst cooling over-night. The next day the cold meat was sliced, the slices were separated with Cellophane and sealed in Cellophane bags. Samples were thawed and tasted cold after 3 and 5 months in the freezer. On removal from the brine the chap was rinsed, hung up to dry for 48 hours and then sealed in a polythene bag and frozen for 8 months before it was tasted hot.

A piece of pork from the belly was pickled in a similar manner, drained, cut into approximately 2 lb. pieces, sealed in polythene and frozen. Packets were removed from the freezer, weighed, thawed in the cold, and cooked at simmering point (190°F.) allowing 25 min. per lb. Pieces were tasted after 4 to 6 weeks freezing and after 7 months.

Results.

Wrapping Materials.

In the tests with fruit or fruit pulp the losses from standard containers stored for periods of up to 6 months in the freezer averaged :—

Polythene (Alkathene), Pliofilm or plastic boxes .. .. .. <0·1%
Cellophane (grade MSAT 300/30) .. .. 0·3%
Waxed cardboard tubs and cartons sold as freezing containers .. 1·5%

Waxed cardboard boxes were found useless for storing semi-liquid foods, as many leaked, and the losses in evaporation in the others was up to 6% over a 3 day period.

Losses of ascorbic acid, an index of the permeability of the materials to air, were :—

Polythene, pliofilm and plastic boxes 12%
Cellophane 16%
Waxed cardboard tubs and cartons 19%

The losses in weight occurring during the storage of pork chops in Cellophane, polythene and greaseproof paper are shown in Table I. The poor protection afforded by greaseproof paper will be noted ; the chops wrapped in this material showed marked loss of weight and typical freezer burn, which may also occur with chicken when the wrapping has been perforated in the freezer (Figs. I and II). Some of the losses recorded for Cellophane- and polythene-wrapped meat were due to faulty seals or to the covers having been pierced by a bone. Great care is needed in handling joints in the freezer as the wrapping material is easily damaged when cold.

TABLE I.

EFFECT OF COVER ON LOSS OF WEIGHT DURING FREEZING.

Product	Method of covering	Fresh weight average (g.)	No. of samples	Length of storage (months)	Moisture loss (% of fresh weight)	
					Range	Mean
Loin	Cellophane	685	4	4–7	0	0·0
		684	2	10	2–3*	2·5
		852	2	12	0–4*	2·0
Chops	Cellophane	163	1	4	4	
		204	3	10–12	0–8*	4·7
	Greaseproof paper	149	1	4	4	
		201	4	5	10–17	14·2
		216	3	10–12	21–23	22·0
	Polythene	234	1	4	0	
		221	4	5	0–0·2	0·1
		216	3	10–12	3–4*	3·3
Sausage meat	Aluminium foil	115	8	3	0–0·3	0·1
		115	6	6	0·6–4·5*	1·7
	Polythene	115	5	6	0	0·0

* Slight perforation of container during storage.

TABLE II.

PALATABILITY OF PORK LOIN AND CHOPS AFTER FREEZING.

Product	Method of covering	Length of storage (months)	Palatability of fat		Palatability of lean	
			Outside	Inside	Outside	Inside
Loin	Cellophane	4	Excellent	Excellent	Excellent	Excellent
		6	Acceptable (4) Poor (2)	Good (5) Poor (2)	Acceptable (4) Poor (1)	Good (5) Poor (1)
		7	Acceptable (7)	Good (7)	Good (7)	Good (7)
		10	Acceptable (4) Slightly rancid (6)	Good (5) Acceptable (5)	Good but tough (10)	Good but tough (10)
		12	Slightly rancid (1) Rancid (9)	Acceptable (2) Poor (8)	Acceptable but tough (5) Poor (5)	Acceptable but tough (10)
Chops	Cellophane	4	Good (7)		Good (7)	
		10–12	Slightly rancid (7)		Slightly rancid (7)	
	Greaseproof paper	4	Poor (7)		Poor and tough (7)	
		5	Rancid (7)		Rancid and tough (7)	
		10–12	Inedible (7)		Inedible and dry (7)	
	Polythene	4	Excellent (7)		Excellent (7)	
		5	Good (7)		Good (7)	
		10–12	Slightly rancid (7)		Slightly rancid (7)	

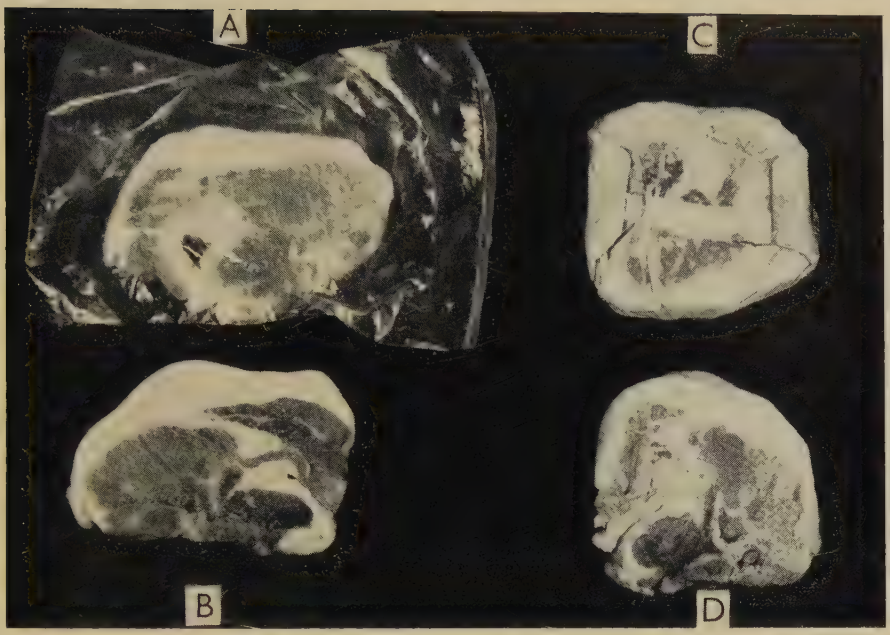


Fig. 1. Frozen pork chops after storage for 3 months.
A. & B. Stored in polythene bags.
C. & D. Stored in greaseproof paper : grey patches on the lean show freezer burn

Fig. 2. Frozen chicken stored in moisture-proof bags.
A. Correctly sealed.
B. Bag torn during storage in freezer : skin showing severe freezer burn.

Storage, Thawing and Cooking Tests.

Palatability tests of roast loins and grilled chops are summarized in Table II, where figures in brackets indicate the number of members of a tasting panel who assigned a particular rating.

It will be seen that where the wrapping material prevented all loss of moisture the palatability was rated excellent after four months' storage. A very small loss from one chop decreased the rating to good, but chops wrapped in greaseproof paper, with a loss of 19% moisture, were rated as poor and tough, and after five months' storage as rancid. After ten months the chops wrapped in greaseproof paper were inedible, whereas those wrapped in polythene or Cellophane were only slightly rancid : the joints were still acceptable except for the outer portion of fat, which could easily be trimmed off. It should be noted that the joints were frozen 48 hours after killing, whereas the chops were frozen five days after killing (3).

The loss from " drip " on thawing did not seem to be related to length of storage or method of thawing, but varied from joint to joint according to the cut and the proportion of fat and bone. The loss on cooking due to evaporation was a little less in the frozen than in the fresh joints but, combined with the losses due to thawing, the totals were very similar. The amount of fat forming dripping during cooking varied considerably from one joint to another. These results are summarized in Table III.

TABLE III.

LOSS OF WEIGHT OF JOINTS OF PORK AFTER FREEZING AND COOKING.

Length of storage	Thawing temperature (approx. °F.)	Fresh weight (g.)	Percentage loss of fresh weight				Total
			Before cooking		After cooking		
			Stored, frozen	On thawing	As dripping	By evaporation	
Fresh (a) ..	—	816	—	—	13·0	11·2	24·2
Fresh (b) ..	—	824	—	—	8·6	17·2	25·8
4 months ..	64	753	0·0	1·9	7·5	11·2	20·6
6 months {	64	789	0·0	0·9	15·2	12·6	28·7
	38	794	0·0	0·0	10·6	10·6	21·2
7 months ..	38	650	0·0	2·4	8·0	12·7	23·0
10 months {	64	703	3·0	0·0	18·0	16·0	37·0
	38	667	2·1	1·5	14·8	10·6	29·0
12 months {	64	950	3·7	2·9	10·4	13·4	29·8
	38	767	0·0	4·6	7·4	10·2	22·2

The losses on cooking skinless sausages, with and without the addition of egg, were as follows :—

No egg	..	21 to 23·5%	..	mean 22·3%
Egg	..	15 to 17%	..	„ 16·2%

TABLE IV.

PALATABILITY OF SAUSAGE MEAT.

Sample	Seasoning	Fresh	Storage period			
			3 months	6 months	9 months	19 months
(a)	None	Acceptable, insipid (8)	Acceptable, insipid (8)	Acceptable, insipid (8)	Acceptable, insipid (8)	Acceptable (5) Slightly rancid (2)
(b)	Salt only	Acceptable (8)	Poor (3) Rancid (5)	Rancid (8)	Inedible (8)	Inedible (7)
(c)	Spices only	Acceptable, lacks salt (8)	Acceptable, lacks salt (8)	Acceptable, lacks salt (8)	Acceptable, lacks salt (8)	Poor (3) Slightly rancid (4)
(d)	Salt and spice	Good (8)	Poor (5) Slightly rancid (3)	Poor (6) Slightly rancid (2)	Poor (5) Slightly rancid (3)	Inedible (7)
(e)	None	Acceptable, lacks salt (8)		Acceptable, lacks salt (8)	Acceptable, lacks salt (8)	
(f)	Salt and spice	Good (8)		Acceptable (8)	Poor (3) Slightly rancid (4)	

Samples (a) to (d) wrapped in aluminium foil; (e) and (f) in polythene.

The effect of salt on rancidity.

Sausage meat. Table IV shows the time elapsing before rancidity was detected in sausages made with and without salt and spices. Where salt was included most members of the panel were able to detect rancidity after three months' storage, but where salt was omitted only two members of the panel could detect rancidity after nineteen months. The addition of spices with the salt so masked or inhibited rancidity that it was not detected by all members of the panel even after nine months' storage.

Pickled Pork. The pickled forehock which had been cooked and sliced before freezing was acceptable after three months' storage, but inedible after five months. A piece of pickled belly frozen raw was rated excellent when cooked after six weeks' storage, but a similar piece stored for seven months had become slightly rancid on the outside, though the inner lean and fat were acceptable. Similarly the outside fat of the chap was distinctly yellow and rancid after eight months' freezing, but the inner lean and fat were considered either acceptable or very slightly rancid.

Summary.

Investigations of the quality of deep-frozen pork and pork products have confirmed American findings and shown that :—

(1) Adequate wrapping of the meat before freezing is essential; polythene, Cellophane (grade MSAT 300/30) and aluminium foil gave good protection, but greaseproof paper was inadequate.

(2) Pork loins and chops, when adequately wrapped, could be kept satisfactorily for seven months, but the outside fat showed slight rancidity after ten months' storage.

(3) Pickled pork, frozen raw, developed slight rancidity of the outer fat after seven months, and frozen cooked slices were inedible after five months.

(4) Sausage meat may have spices added, but salt should be omitted before freezing, as it hastens the onset of rancidity.

(5) The loss in moisture due to " drip " on thawing joints of pork is compensated by less evaporation during cooking, so that the total loss of weight on cooking frozen and fresh pork is similar.

REFERENCES.

(1) Mackey, A. O., Oliver, A. W. and Fang, S. C. (1952) *Food Res.*, **17,** 409.
(2) Noble, I. and Hardy, F. (1945). *Food Res.*, **10,** 165.
(3) Tressler, D. K. and Evers, C. F. *The Freezing Preservation of Foods.* Avi Publishing Co. Inc., New York (1947), (a) p. 231; (b) p. 252; (c) p. 500.
(4) Wiesman, C. K. and Ziemba, J. V. (1946). *Food Inds.*, **18,** 1863.
(5) Chang, I. and Watts, B. M. (1950). *Food Res.*, **15,** 313.

A RAPID METHOD OF ASSESSING BLACK CURRANT LEAF SPOT.

By G. M. CLARKE and A. T. K. CORKE.

In recent years precise methods have been sought for assessing the amount of leaf spot infection in experimental plantations of black currants. In 1953 and 1954, Corke (1, 2) counted the numbers of infected and uninfected leaves on a number of shoots in each experimental plot ; it was shown that the most precise assessments by this method would be obtained by examining a small number of shoots on each of a large number of bushes (1). The ultimate extension of this would be to assess the percentage area of each leaf affected on a single shoot on every bush in an experimental plantation, but such a time-consuming method would be impracticable.

A method enabling records to be taken throughout the season from every experimental bush was therefore planned for 1955 and is described in this paper. The method was rapid, and so reduced personal errors due to fatigue ; interruptions by the weather were also less important. Precise results were obtained at regular intervals throughout the season from estimations of the amount of leaf spot on large numbers of complete bushes.

Experimental.

Site. An acre of black currant bushes of the variety Baldwin was divided into 24 plots, randomised for 3 spraying treatments. Each plot consisted of 4 rows of 11 bushes, of which 9 bushes in each of the middle two rows were assessed, leaving the remainder as guard bushes.

An adjoining acre of the variety Seabrook's Black was divided into 18 plots each of 20 bushes, for comparison of 2 cultivation treatments.

Method of assessment. Visual estimates of the amount of infection, expressed as the percentage leaf area affected on a whole bush, were made at intervals of three weeks commencing with the appearance of leaf spot : on June 24th for the variety Baldwin and July 6th for Seabrook's Black. The bushes to be assessed were compared with diagrams showing 5 levels of infection of a bush (10, 25, 50, 75 and 100%) superimposed on 6 levels of foliation (100, 80, 60, 40, 20 and 10%). The resulting diagrams, showing the percentages of the total leaf area affected and unaffected by infection and defoliation, were grouped into 6 categories (Table I).

TABLE I.

Percentage total leaf area affected	10	25–28	40–55	60–75	80–90	91–99
Assessment category	5	25	40	60	80	95

In Table I the figures for percentage total leaf area affected were obtained by subtracting from 100 the percentage total leaf area remaining unaffected. This unaffected area is the product of percentage foliation and percentage existing leaf area uninfected.

The intervals between adjacent categories were chosen to be of the minimum size easily distinguishable in the field, but still sufficiently small to yield good results (Fig. 1).

Results.

In the dry season of 1955, leaf spot progressed quite slowly, and the greatest variability, which occurred when the average leaf area affected was about 50%, was not reached on the variety Baldwin until September 14th. On this occasion a difference of about 15% in the mean leaf area affected was needed for a difference between two treatments to be detected statistically (see Discussion below). This represents an average difference of about one whole category. Assessments made on June 24th and October 7th, when the mean leaf areas affected were 10% and 60% respectively, were precise enough for differences of 7% to 10% to be detected.

The disease spread more slowly and more regularly on the less susceptible variety Seabrook's Black. The assessments showing the greatest variation in the amount of leaf spot present on this variety were obtained on September 28th. In order to compare these results with those obtained with Baldwin, precision was calculated as if there had been three treatments. Under these circumstances, a difference of 10% in the mean leaf area affected would have been detectable. For the two treatments actually compared, a difference of 8% in the mean leaf area affected was detected at this time.

The degree of precision obtained was satisfactory, because all the treated bushes were assessed with reasonable accuracy and it has been shown that the greatest contributor to loss of precision is between-bush variation (1).

Discussion.

When two or more experimental treatments are being compared for their effect as inhibitors of leaf spot, the mean percentage leaf area affected is calculated for each treatment. A statistical "significant difference" is also calculated; this is the least amount by which the means must differ in order that their difference in effect can be ascribed with reasonable certainty to the treatments rather than to natural variation between plots of bushes. The size of this significant difference is directly proportional to the square root of the variance; the most precise assessment of percentage leaf area affected in an experimental plot is that which is subject to the smallest variance (1). Hence the more precisely an assessment can be made, the smaller is the difference between means that can be detected statistically.

Assessments made on the plantation of Baldwin in 1954 and 1955 and on Seabrook's Black in 1955 were available for statistical analysis. It was previously shown that the assessments of percentage of leaves infected in 1954 would have been more precise if they had covered more bushes per plot (1). Such results would have been capable, with experimental plots of the existing size (18 recorded bushes), of yielding figures sufficiently precise for two treatments to be statistically different if the mean infection differed by about 8%, a difference which would certainly not be discerned by visual inspection. The 1954 figures were those obtained on July 29th, and were the most variable (i.e. the least precise) of the season; early counts on individual bushes were all small (0% to 10%), and late-season counts were all high (90% to 100%).

Fig. 1. FIELD ASSESSMENT DIAGRAM FOR BLACK CURRANT LEAF SPOT.
Categories for assessment of percentage total leaf area affected on a bush.
(White areas represent leaf area remaining unaffected).

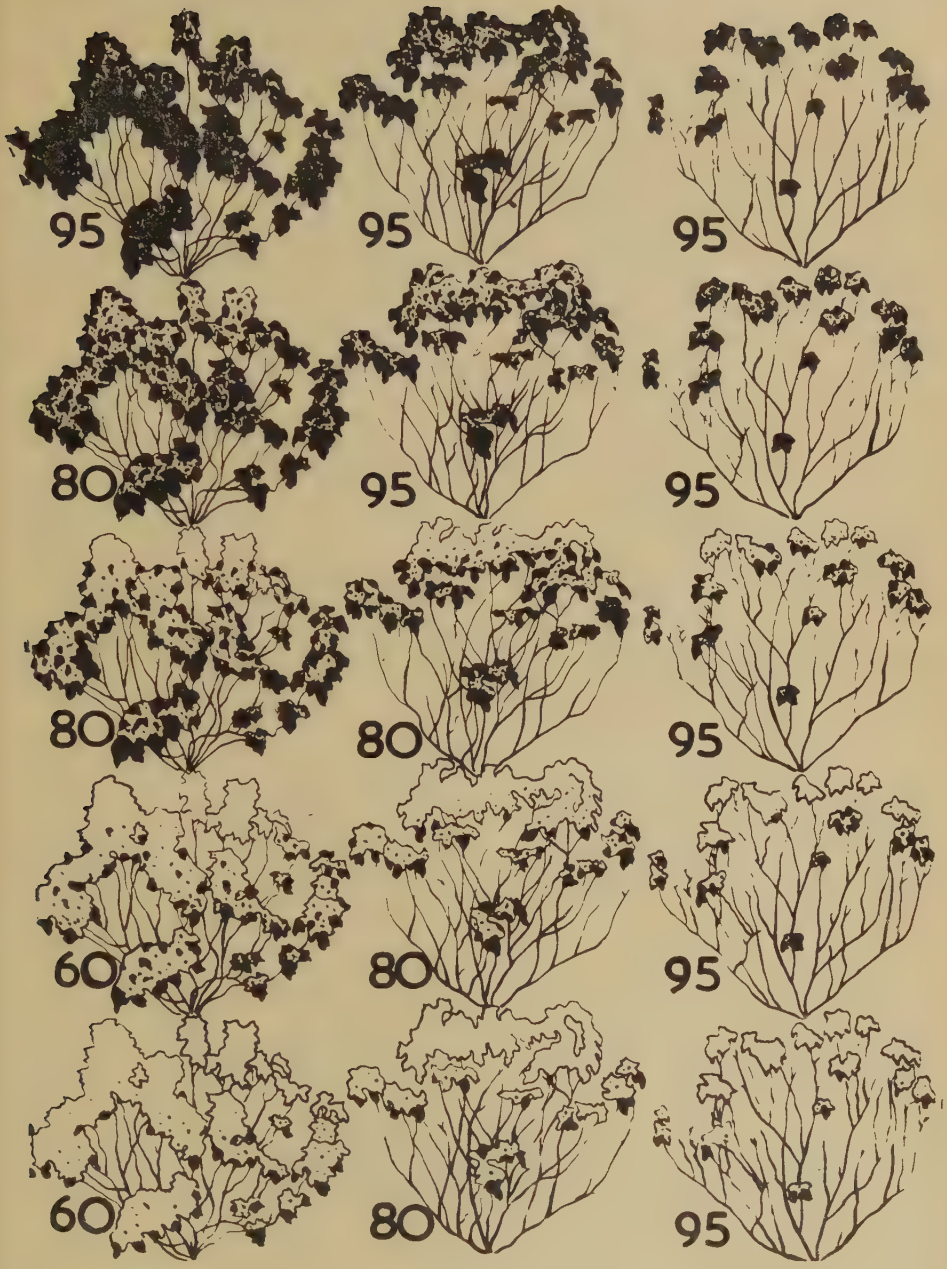


Fig 1. FIELD ASSESSMENT DIAGRAM FOR BLACK CURRANT LEAF SPOT.
Categories for assessment of percentage total leaf area affected on a bush.
(White areas represent leaf area remaining unaffected).

On both these occasions there was less variation between the counts, leading to more precise results. However, because insufficient information had been available concerning sources of variation, the optimum precision was not reached, a difference of about 12% being needed for statistical detection. It is shown above that in 1955 the corresponding difference was 15%. The method of assessment used in 1955 therefore gave results of comparable precision.

The method of assessment described above was used on the same plantation in 1955, on unit plots of the same size. The assessments required at least 50 man-hours in 1954, compared with a maximum of 10 man-hours in 1955, with no appreciable loss of precision. Also, the possibility of using two observers enabled independent estimations to be made from both sides of a bush at the same time, a necessary safeguard in recording by a method that involves eye-estimation.

In an exact statistical analysis such as that carried out on the results described above, percentage figures are " transformed " before computations are carried out. However, if an arithmetic mean of percentage leaf area affected is calculated from the actual assessment figures, this is a good approximation to the best statistical estimate of the area affected. A good working rule for comparing two treatments is as follows : if about 150 to 200 bushes of a given variety can be examined under each treatment (taking care to record objectively, in ignorance of the actual distribution of the treatments), and the mean areas of leaf affected differ by 10% or more, such a difference is statistically significant. This is likely to err on the conservative side for varieties not very susceptible to the disease, where the spread tends to be more regular.

Summary.

A rapid method, used at Long Ashton in 1955, is presented for assessing the percentage leaf area affected by leaf spot in a black currant plantation. The method is shown to yield results of good precision, detecting statistically quite small differences between differently treated groups of bushes. A working rule is suggested for estimating the significance of such differences with little calculation.

Acknowledgments.

The authors wish to thank Miss M. E. Holgate for carrying out the statistical analysis, Miss N. M. Waugh for preparing the assessment diagrams, and Miss M. Marlow and Miss M. Waller for assistance in making field records.

REFERENCES.

(1) Clarke, G. M. and Corke, A. T. K. (1955). Sources of variability in sampling black currants for leaf spot. *Ann. Rep. Long Ashton Res. Sta.*, 1954, 199–201.

(2) Corke, A. T. K. (1954). Black currant leaf spot : I. Studies of perennation and infection. *Ann. Rep. Long Ashton Res. Sta.*, 1953, 154–158.

METEOROLOGICAL RECORDS, 1955

Month	Air Temperature (°F.)							Rainfall (″)			Sunshine (hours)		Soil Temperature at 09.00 G.M.T.		
	Means		Means of A & B	Diff. from normal	Max.	Min.	No. of ground frosts	Total	Per cent. of average	Most in a day	Daily mean	Per cent. of average	4″	8″	24″
	A Max.	B Min.													
January	43·1	33·4	38·3	—2·1	53	19	15	3·38	100	0·76	0·95	61	37·8	38·9	38·9
February	40·7	30·9	35·8	—4·9	51	20	23	1·78	69	0·47	2·47	102	36·1	37·2	40·9
March	46·6	31·0	38·8	—5·1	59	22	26	1·27	54	0·35	4·96	127	36·5	37·1	39·1
April	57·4	41·2	49·3	+1·7	66	28	11	1·42	61	0·49	5·72	104	47·7	46·9	46·9
May	57·5	42·9	50·2	—2·9	69	35	8	3·91	151	0·69	6·88	109	51·7	50·2	52·1
June	65·5	50·4	57·9	—0·6	75	37	1	2·59	123	0·93	5·00	71	58·8	57·7	57·8
July	75·4	54·7	65·1	+3·5	85	44	0	0·15	5	0·10	8·74	138	66·1	64·9	62·8
August	76·3	55·8	66·1	+5·1	89	43	0	0·64	18	0·24	6·57	111	65·3	64·9	63·5
September	67·4	49·7	58·5	+1·4	75	41	1	1·34	43	0·30	5·21	111	58·4	58·3	61·0
October	57·5	42·0	49·7	—1·4	69	26	13	1·42	37	0·60	3·65	112	49·5	50·6	55·6
November	51·1	38·0	44·6	—0·2	61	24	17	4·22	114	1·31	1·62	86	43·6	44·9	49·7
December	49·8	37·5	43·7	+2·3	56	26	18	3·71	104	0·63	0·73	50	42·7	43·5	46·7
Totals or Means ..	57·4	42·3	49·8	—0·3				25·83	71		4·39	105	49·5	49·6	51·3

G. E. Clothier.

FACTORS AFFECTING THE ACTIVITY OF NITRATE REDUCTASE IN CAULIFLOWER PLANTS.

By E. J. HEWITT, E. G. FISHER and M. I. CANDELA.

The presence and function of molybdenum in nitrate reductase enzymes isolated from micro-organisms and from higher plants, represented by soybean, has been established (1, 2). This enzyme in cauliflower shows wide differences in apparent activity. It is desirable to elucidate the factors responsible as they may also be concerned in the interpretation of other aspects of the role of molybdenum in plant physiology. The preliminary observations reported here concern the apparent distribution of the enzyme in relatively mature plants and the effects of nutrition and some environmental factors on its activity. Some tests were also made on the activity of acid phosphatase in order to compare its extraction with that of nitrate reductase.

Experimental.

Cauliflower plants var. Majestic were grown in sand culture by methods already described (3). Molybdenum was provided at approximately 0·00002 p.p.m., 0·05 p.p.m. and 10–20 p.p.m. Nitrogen was given either as nitrate, nitrite or ammonium sulphate.

The enzymes were extracted under standard conditions by hand grinding with neutral, acid-washed silica sand for 15 minutes in a chilled mortar. In 1955 0·1M phosphate buffer pH 8·8 at 0°C. which contained 10^{-3}M cysteine and 10^{-3}M ethylenediaminetetraacetic acid (EDTA) (2) was used as the extracting reagent. In 1954, the extraction was made with 0·1 M phosphate buffer pH 7·0 in the presence of 10^{-4} M cysteine and EDTA. The cell fluid obtained by extracting tissues with three times their weight of reagent was squeezed through muslin and centrifuged at 15–18,000 × g. at 0°C. 0·2 or 0·5 ml. of the cell-free supernatant fraction was used almost immediately in the following mixture :—

0·5 ml. extract, 1·0 ml. 5 per cent. sodium nitrate, 0·1 ml. reduced co-enzyme I (DPNH 1 mg./ml.), 0·05 ml. boiled pig heart extract, 2·5 ml. 0·2 M phosphate buffer pH 7·8, 1·0 ml. water.

The mixture was incubated at 27°C. for 10 minutes in 1955, or 20 minutes in 1954 after equilibration of the non-enzymatic components. Activity was measured in terms of nitrite production (4) and expressed as millimicromoles of nitrite (mμM NO_2^-) per minute per milligram protein for specific activities and as mμM NO_2^- per 10 minutes per 0·2–0·5 ml. extract for relative total activities. The reaction rates were linear over the duration of the assays. Protein content was estimated turbidimetrically after precipitation by trichloracetic acid in 1955 and by the biuret reaction in 1954 (5).

Suitable blank determinations were made in the absence of DPNH, nitrate or enzyme extract and all estimations were carried out at least in duplicate with satisfactory agreement. The presence of nitrite contamination in the nitrate solution was eliminated by the addition of 0·1 ml. 10^{-5} M NH_4Cl solution to 100 ml. of 5 per cent. $NaNO_3$ followed by boiling for about three minutes. The treated solution then contained a negligible concentration of nitrite.

It was often impossible to carry out a complete set of determinations on the same day and single plants or parts thereof were chosen as daily units. Comparisons between nutritional treatments were therefore unavoidably separated by an appreciable interval in time in some of the experiments. In some experiments the tissues were frozen to −18°C. for 24 hours before extraction, and in others fresh tissues were used immediately after removal from the plant, but all comparative tests employed the same procedure. Most of the results reported here were obtained during late autumn, winter and early spring, when growth was relatively slow. In 1954 the foliage was used as a whole except the two oldest leaves and young leaves less than two centimetres in length which were rejected. In 1955 plants were sampled in relation to nodes or morphological regions.

Results.

Distribution of net activity (Figs. 1 and 2).

Plants grown in 1955 with nitrate were divided into roots, stems, petioles and leaves. The last three regions were sampled in relation to the number of nodes on the plants.

Roots. No net activity of nitrate reductase was detected in roots examined on several occasions. Nitrite reductase was found to be active in these tissues. Tentative estimates of nitrite loss with and without the addition of nitrate indicated relatively low nitrate reductase activity in roots.

Leaf laminae. In plants given nitrate with molybdenum the net total nitrate reductase activity in laminae was greatest in mature, fully expanded leaves adjacent to the oldest ones ; two or three of these leaves remained on the plant at the time of sampling. Activity decreased progressively in younger leaves. Nitrite reductase was correspondingly relatively low in the foliage but the distribution has not yet been determined.

The distribution of activity in leaves of plants given nitrite or ammonium sulphate was similar to that in leaves of plants given nitrate.

Petioles and stems. The distribution of nitrate reductase activity in petioles was mainly similar to that in leaves, but in stems the maximum activity occurred in relatively young regions.

Specific activity and protein. The specific activity of nitrate reductase was much greater in extracts from petioles than from leaves, and was also relatively high in parts of the stem. The distribution of total extractable protein was quite different from that of nitrate reductase : it increased progressively from the oldest to the youngest nodes.

Age of plant. Leaves of plants aged 20 weeks, grown with 10 20 p.p.m. Mo in 1954 with each of the three sources of nitrogen, contained considerably less total activity than those of plants aged 12 weeks. The specific activities for each respective nitrogen source were about the same for the two age groups (Table I).

Effects of nitrogen source and molybdenum level (Figs. 2 and 3. Tables I and II).

Nitrate reductase could be detected in all aerial parts when molybdenum was given, but the activity was always lower in plants given ammonium sulphate than in those given nitrate or nitrite. In 1954, when plants were

given 10–20 p.p.m. Mo, the total and specific activities at 12 weeks, associated with these sources of nitrogen, decreased in the order nitrate> nitrite> ammonium sulphate and differed significantly from each other ($P < .001$). A high level of nitrate produced higher total activity but the same specific activity as that of plants given the normal level.

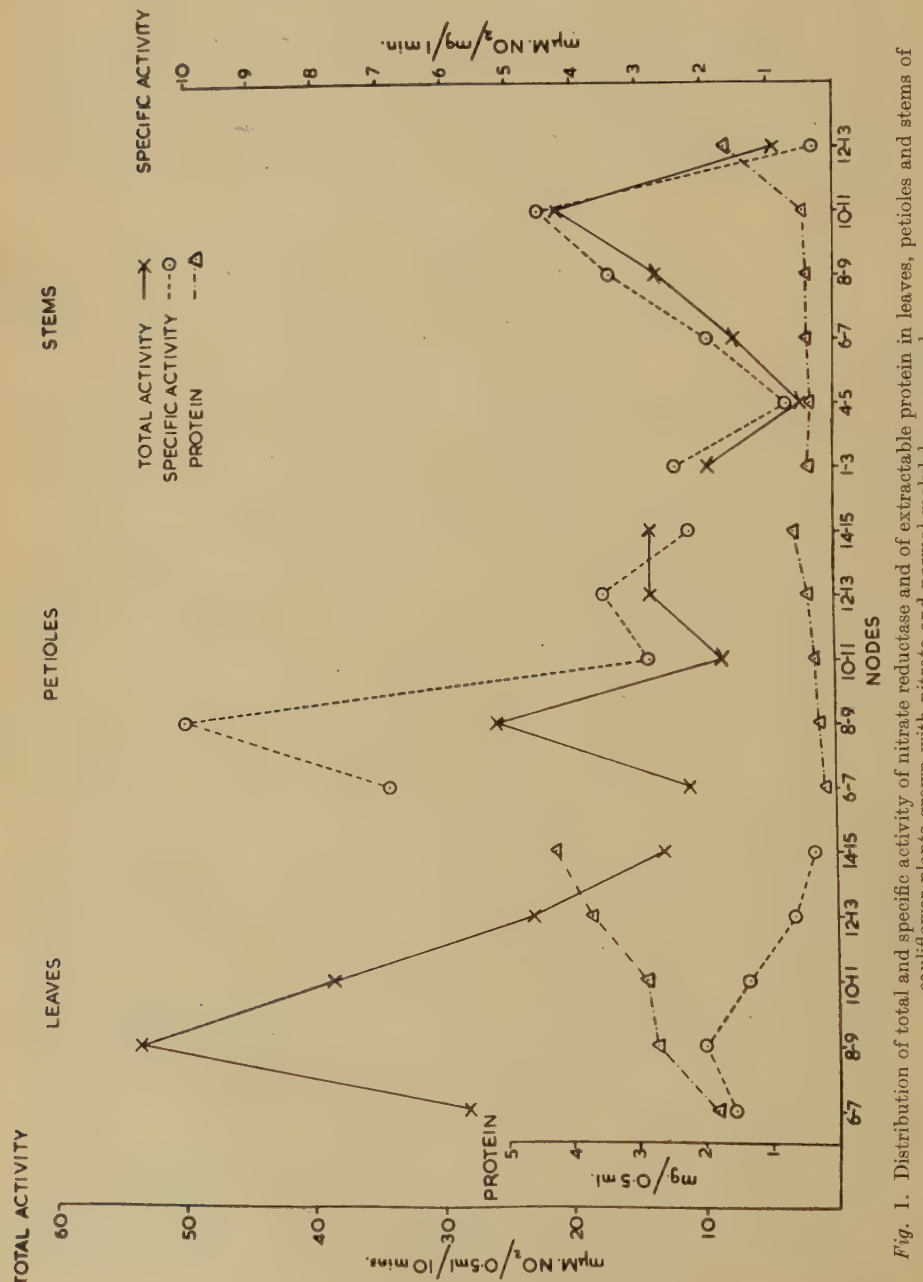


Fig. 1. Distribution of total and specific activity of nitrate reductase and of extractable protein in leaves, petioles and stems of cauliflower plants grown with nitrate and normal molybdenum supply.

TABLE I.

EFFECTS OF NITROGEN SOURCE AND AGE OF PLANTS ON NITRATE REDUCTASE ACTIVITY IN CAULIFLOWER IN 1954.

Nitrogen supply	Nitrate			Nitrite		Amm. sulph.	
	normal level		double level				
Age of plants : weeks	12	20	20	12	20	12	20
Total activity 0·2 ml. mμM NO_2^-/10′	21·6	12·5	17·7	15·8	13·5	10·4	5·9
Protein mg./0·5 ml.	3·2	2·3	3·2	3·4	2·8	3·0	1·7
Specific activity mμM NO_2^-/mg./1′ ..	1·69	1·36	1·38	1·12	1·20	0·86	0·87

TABLE II.

EFFECTS OF 48 HOURS EXCLUSION OF AIR ON NITRATE REDUCTASE ACTIVITY IN CAULIFLOWER IN 1954.

Nitrogen supply	Nitrate		Nitrite		Amm. sulph.	
Treatment	Air	Nitrogen	Air	Nitrogen	Air	Nitrogen
Total activity 0·2 ml. mμM NO_2^-/10′	22·0	11·1	18·5	8·0	9·0	8·0
Protein mg./0·5 ml.	3·1	3·6	3·4	2·8	3·4	3·9
Specific activity mμM NO_2/mg./1′ ..	1·77	0·77	1·40	0·71	0·66	0·45

In 1955 molybdenum was added at 0·05 p.p.m. or was present at a deficiency level of approximately 0·00001 -0.00002 p.p.m. In the plants given nitrate or nitrite the total activities were very low in the molybdenum deficient plants compared with those observed in normal plants in which the total activities were high and similar for both nitrogen treatments. The effect of molybdenum was smaller in plants given ammonium sulphate as the total and specific activities were low in the normal plants.

In the molybdenum deficient plants the total net activity tended to reach a maximum in leaves that were chronologically younger than those showing maximum activity in the corresponding control plants. The apparent effect of molybdenum supply varied greatly according to the region of the plant that had been sampled, and the differences between normal and deficient plants were often relatively small in the youngest and oldest leaves. In molybdenum deficient plants given nitrate the specific activity was higher than in plants given nitrite or ammonium sulphate. These differences in specific activity in molybdenum deficient plants were due to the different effects of molybdenum supply on the total extractable protein of plants given the different nitrogen treatments (Figs. 2 and 3). Thus there was little or no effect of molybdenum in plants given nitrite or ammonium sulphate, but a marked decrease was observed in molybdenum deficient plants given nitrate.

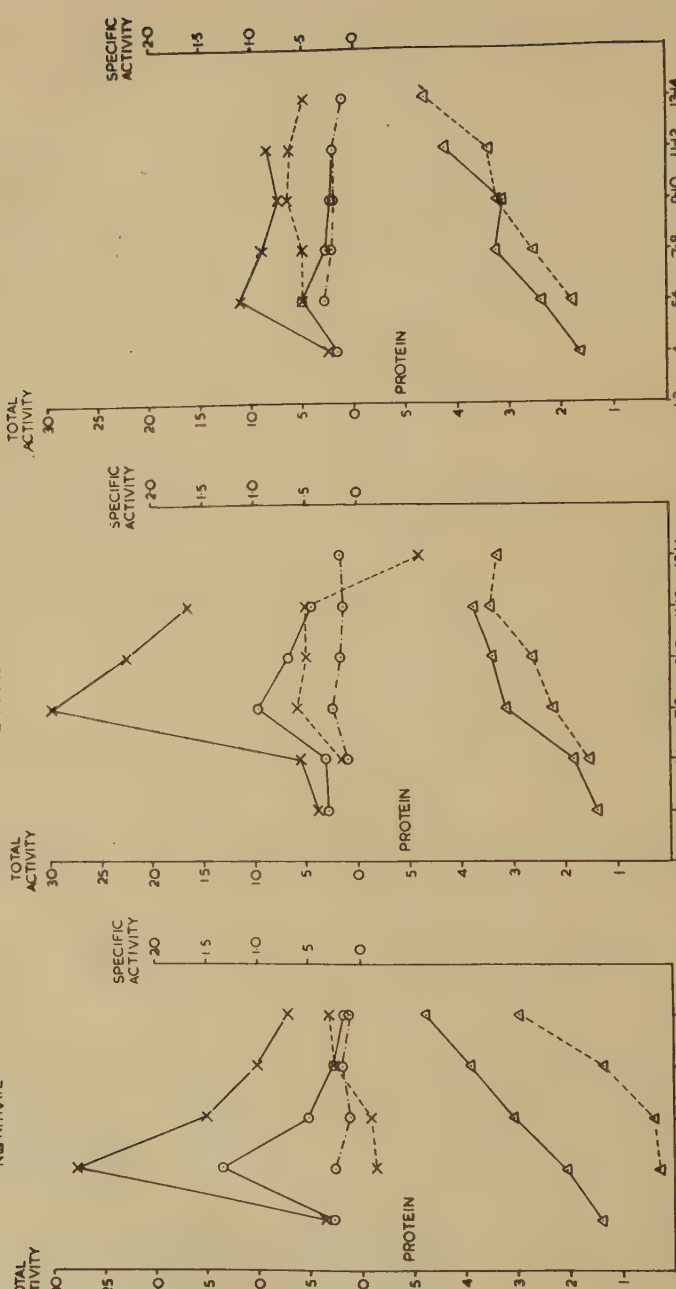


Fig. 2. Distribution of total and specific activity of nitrate reductase and of protein in leaves of cauliflower grown with different sources of nitrogen at normal or deficiency levels of molybdenum.
——— +Mo ; - - - - - -Mo.

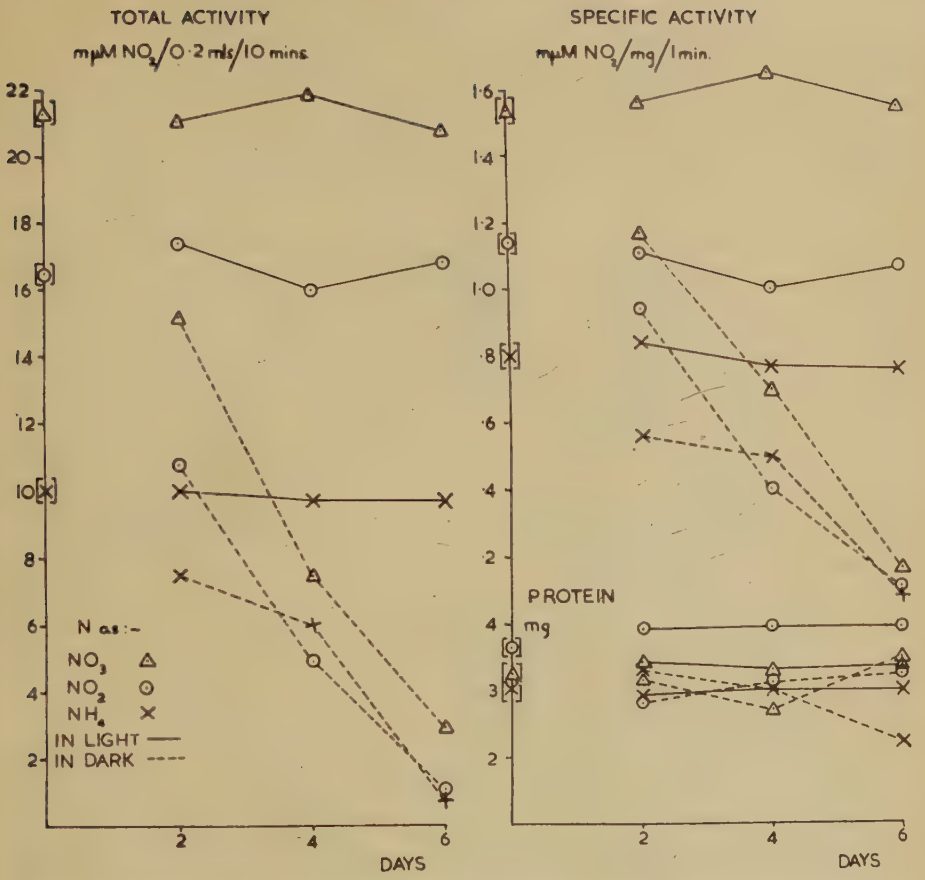


Fig. 3. Effects of light and darkness on total and specific activity of nitrate reductase and on protein content of leaves of cauliflower grown with different sources of nitrogen and high level of molybdenum.

The points shown in brackets on the ordinates represent the mean values of 6 estimations made on similar plants at the beginning of and immediately after each experiment.

Effect of molybdenum infiltration on nitrate reductase and acid phosphatase activities. (Fig. 4).

Leaves of molybdenum deficient plants grown with nitrite were divided into opposite halves, weighed and infiltrated several times under vacuum with either water or 10^{-5}M molybdate solution until the tissues were apparently water-soaked. The treated half-leaves were kept in covered jars in a saturated atmosphere for periods up to 24 hours, during which they remained turgid and eliminated most of the excess water by transpiration.

The effects on nitrate reductase of molybdenum infiltration at 10^{-5}M were small and not consistent even after intervals of 24 hours. Acid phosphatase activity, which was distributed quite differently from nitrate reductase, was also not affected consistently by molybdate infiltration at the concentration used. The activity was usually similar in both control and molybdenum infiltrated leaves and the distribution resembled that of total extractable protein.

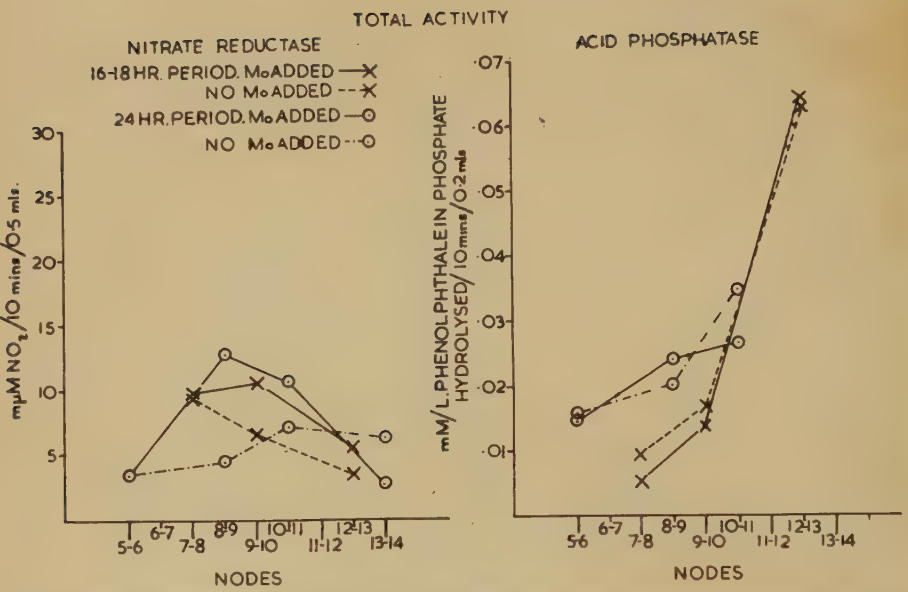


Fig. 4. Effects of infiltration by water, or 10^{-5}M molybdate, of molybdenum deficient leaves of plants grown with nitrite, on distribution and total activity of nitrate reductase and acid phosphatase in cauliflower.

Effects of darkness and anaerobic conditions on nitrate reductase activity.

Plants aged about 13 weeks that had been grown with nitrate, nitrite or ammonium sulphate were kept in darkness in the greenhouse or were allowed to grow in the normal way. Total nitrate reductase activity in the foliage was determined at 2 day intervals (Fig. 3). Plants kept in darkness showed marked and progressive decreases in total and specific activities, which fell to approximately one-tenth or less of those in control plants after 6 days. The total extractable protein did not, however, fall in a corresponding manner. The differences were highly significant at all times ($P < \cdot001$).

Young plants grown with the three sources of nitrogen were enclosed in transparent plastic bags and maintained in an atmosphere of nitrogen for 48 hours in the greenhouse without excluding light. Nitrate reductase total and specific activities are shown in Table II ; they usually decreased greatly under anaerobic conditions but total protein was not affected in a corresponding way. These changes in activity were significant when tested in relation to the rates of reaction.

Discussion.

The relative effects of molybdenum supply and of source of nitrogen on the net total, and specific nitrate reductase activities depended greatly upon the region from which samples were taken and upon the developmental stage. Although the greatest differences due to changes in these factors usually occurred in mature leaves just above those bordering on senescence, the precise nodes at which such maximum differences were observed could not be forecast with sufficient certainty to ensure the maximum contrast by sampling from a single region.

The total activities observed by Evans and Nason (4) in the leaves of potato, barley and musk melon were substantially less than that found in cauliflower when re-calculated on a comparable basis of 14 per cent. dry weight in fresh tissue. Highest activity in soya bean was found by these workers in primary leaves, young trifoliate leaves and meristematic leaves of young plants. This was also greatly in contrast with results obtained with cauliflower, where mature leaves of plants, which were several weeks old, contained the highest net activities.

It was apparent that both the source of nitrogen and the level of molybdenum were major factors in determining the total and specific activities. The effects of omission of molybdenum on the total and specific activities of the enzyme clearly show an effect of molybdenum on the *production* of the enzyme by cauliflower tissues in a manner similar to that observed in *Neurospora* (6). The failure of Evans and Nason (4) to observe a consistently comparable effect in soya bean was probably due to the molybdenum content of the seed, which is adequate in many large seeded legumes for the growth period of 30 days that they used. The adaptive nature of the enzyme in higher plants had not been established at that time (4) but the large differences in activity due to nitrate or ammonium nutrition indicate an adaptive character. The significance of high activities in the presence of nitrite and the definition of adaptation are beyond the scope of the present discussion.

Plants grown with nitrite, however, present points of particular interest since they resemble those grown with nitrate in regard to their relative nitrate reductase activity and to the effects of molybdenum supply on the magnitude of their nitrate reductase activity ; on the other hand, they resemble those grown with ammonium sulphate in regard to the effects of molybdenum on total protein. The differences between the type of response shown by plants given each of the three sources of nitrogen should provide the means not only to distinguish between adaptive responses to molybdenum or nitrogen source as such, but also to study the extent to which the capacity for fresh protein synthesis may control the rate of such adaptive responses.

Plants grown with nitrite were chosen for the first infiltration tests as it was then thought that their ability to produce high nitrate reductase activity when grown with molybdenum, combined with their abundant leaf protein under molybdenum deficiency conditions, would make them ideal subjects in which to observe early effects of the introduction of molybdenum. However, in plants already provided with abundant protein, as for example those given nitrite, the synthesis of new protein to form a specific enzyme, i.e. nitrate reductase, may be relatively slow in spite of the adaptive stimulus produced on the introduction of molybdenum. This possibility may account for the poor response in the plants given nitrite compared with that inferred to occur in plants given nitrate (8) and is under further investigation.

The marked decreases in activity of nitrate reductase under prolonged darkness or anaerobic conditions, without corresponding changes in total protein, indicate that selective inactivation or loss of certain enzymes may occur in unfavourable physiological environments. This effect contrasts with the persistence of other enzymes, e.g. invertase and phosphatase, in detached leaves during progressive proteolysis (7) in the dark, and also with the tendency for the specific activity of nitrate reductase to maintain a normal level in older plants where total extractable protein has declined.

Acknowledgments.

The tenure of an overseas studentship of the Consejo Superior de Investigaciones Scientificas, Madrid, by M.I.C. in 1954–55, and the grant of Sabbatic Leave in 1955 to E.G.F., which respectively enabled them to participate in this work, are gratefully acknowledged. We wish to thank Mr. G. M. Clarke for statistical analysis of the data for 1954.

REFERENCES.

(1) Nicholas, D. J. D. and Nason, A. (1954). Molybdenum and nitrate reductase : II. Molybdenum as a constituent of nitrate reductase. *J. biol Chem.*, **207,** 353.

(2) Nicholas, D. J. D. and Nason, A. (1955). Role of molybdenum as a constituent of nitrate reductase from soy bean leaves. *Plant Physiol.*, **30,** 135.

(3) Hewitt, E. J. (1952). *Sand and water culture methods used in the study of plant nutrition.* Commonwealth Bureau of Horticulture. Tech. Comm. 22. East Malling.

(4) Evans, H. and Nason, A. (1953). Pyridine nucleotide nitrate reductase from extracts of higher plants. *Plant Physiol.*, **28,** 233.

(5) Robinson, H. W. and Hogden, C. G. (1940). The biuret reaction in the determination of serum proteins. *J. biol. Chem.*, **135,** 727.

(6) Nicholas, D. J. D., Nason, A. and McElroy, W. D. (1954). Molybdenum and nitrate reductase : I. Effect of molybdenum deficiency on the *Neurospora* enzyme. *J. biol. Chem.*, **207,** 341.

(7) Axelrod, B. and Jagendorf, A. T. (1951). The fate of phosphatase, invertase and peroxidase in autolysing leaves. *Plant Physiol.*, **26,** 406.

(8) Ducet, G. and Hewitt, E. J. (1954). Relation of molybdenum status and nitrogen supply to respiration in cauliflower leaves. *Nature*, **173,** 1141.

THE DESTRUCTION OF ASCORBIC ACID BY TISSUE PREPARATIONS OF CAULIFLOWER GROWN WITH DIFFERENT NITROGEN AND MOLYBDENUM SUPPLIES.

By E. J. HEWITT.

The consistently decreased concentrations of ascorbic acid found in molybdenum deficient plants (2, 3, 5) suggest either that the production of ascorbic acid from precursors or its maintenance from dehydroascorbic acid is decreased, or that its rate of destruction in the cell is increased, by molybdenum deficiency. It has been suggested recently that ascorbic acid may be related to cell growth or cell wall extension (13), and it is of interest, therefore, to examine the relation, if any, between ascorbic acid metabolism and the production of whiptail, which in its earliest stages may possibly involve a cell wall separation (1). Ascorbic acid oxidase also appears to be insolubly attached to cell wall residues (7, 12).

Mineral deficiencies are known to produce significant increases in the activity of several enzymes (9, 10, 11). The possibility of increased destruction by oxidative enzymes in molybdenum deficient tissues was chosen for initial study as information was already available regarding ascorbic acid oxidase activity in cell wall residues (7, 12), and the other oxidative enzymes that might be involved are relatively well known in many plant tissues.

Experimental.

Cauliflower plants var. Majestic were grown in sand culture at normal or deficiency levels of molybdenum with nitrogen given as nitrate, nitrite or ammonium sulphate, as described elsewhere (3, 4).

Three general types of tissue preparations were used :—

(i) Small fragments of unbroken tissue were frozen at —18°C. for some hours and packed whilst still frozen into small heavy-walled glass tubes which measured about 60 × 4 mm. A short, loose-fitting glass plug was placed on top and the contents of the tube were thawed for a few minutes. The tube was centrifuged at 15,000 × g. at 0°C. for 15 minutes and the clear, pale amber supernatant fluid was withdrawn with a needle pipette from which it was transferred to a small tube immersed in ice.

Tissues weighing 100–300 mg. yielded up to 70 per cent. of their weight as fluid, which was estimated to comprise about 80 per cent. of the water content of the fresh tissues.

(ii) Cell-free extracts of soluble enzymes that were assumed to be free from mitochondria or chloroplasts were also obtained by the usual method of centrifuging (at 18,000 × g. and 0°C.) suitable extracts of ground tissues. The grinding was performed at 0°C. in close fitting rotary glass macerators, usually in the absence of an abrasive.

(iii) Cell wall preparations from samples which weighed 200 mg. or more, and the residual supernatant fractions, were obtained by grinding tissues as described and centrifuging the suspension at 250–400 × g. for 5 minutes.

The supernatant, which contained chloroplast particles and mitochondria, was decanted and the cell wall material was washed twice by resuspending it in water and centrifuging at 500 × g. These cell wall preparations were practically free from contamination by chlorophyll-containing residues. The suspensions were stored in ice until required and appeared to be relatively stable under these conditions.

The activity of enzymes that destroy ascorbic acid was measured in two ways according to the type of preparation. The activity of cell sap produced from frozen tissues was measured at pH 5·0-5·5 by the rate of decrease in optical density at 265 mμ on addition of 40 mg. of the cell sap to a solution which contained about 30 μg./ml. ascorbic acid. The activities of cell wall suspensions corresponding to approximately 30-70 mg. original fresh tissue per 1 ml., or of the other extracts, were measured by the rate of decrease in optical density at 245 mμ of samples of the reaction mixture at pH 6·5 drawn at one minute intervals, killed in metaphosphoric acid, and centrifuged at 2,000 × g. All assays were made at 27°C.

Results.

Cell sap from frozen tissues.

The first series of tests was made on leaf tissues from plants aged 6–7 weeks that were frozen for periods between two and eight days. Plants grown in the molybdenum deficiency treatments at this stage showed the primary symptoms in the presence of nitrate (1), but had not developed whiptail symptoms, which appeared about 1–2 weeks later. The mean results (Table I) for 7 replicates show a marked increase in activity in preparations from molybdenum deficient leaves, significant at the 0·1 per cent. level. In the molybdenum deficiency treatment there was usually more activity in preparations from plants grown with ammonium sulphate or nitrite than in those from plants grown with nitrate, possibly because of the greater protein content of the first two types of plants (6). In plants given molybdenum no differences in activity due to different nitrogen supplies were observed. The rate of reaction was usually linear for several minutes as shown in Fig. 1. The rates of loss of ascorbic acid calculated on the assumption of 85 per cent. water content of the tissues ranged from 45-150 μg./minute/g. fresh weight with an average of 90·5.

TABLE I.

EFFECTS OF MOLYBDENUM AND NITROGEN SUPPLIES ON THE RELATIVE ACTIVITY OF CELL SAP PREPARATIONS FROM FROZEN TISSUES IN DESTRUCTION OF ASCORBIC ACID.

Nitrogen supply	Nitrate		Nitrite		Amm. sulphate	
Molybdenum supply	+Mo	—Mo	+Mo	—Mo	+Mo	—Mo
Mean decrease in optical density at 245 mμ in 10 min. with 40 mg. cell sap	0·487	0·630	0·436	0·794	0·473	0·864
μg. ascorbic acid destroyed per minute per 1 g. fresh weight	73·0	94·5	65·4	109·1	71·0	129·6

Areas of lamina (weighing about 20 mg.) that showed the first signs of whiptail symptoms (1) were taken from plants aged 10–18 weeks. Similar samples were taken from apparently healthy areas of the same leaves and from corresponding areas of leaves on plants given molybdenum. In order to obtain sufficient material for use, the samples were accumulated at —18°C. for two

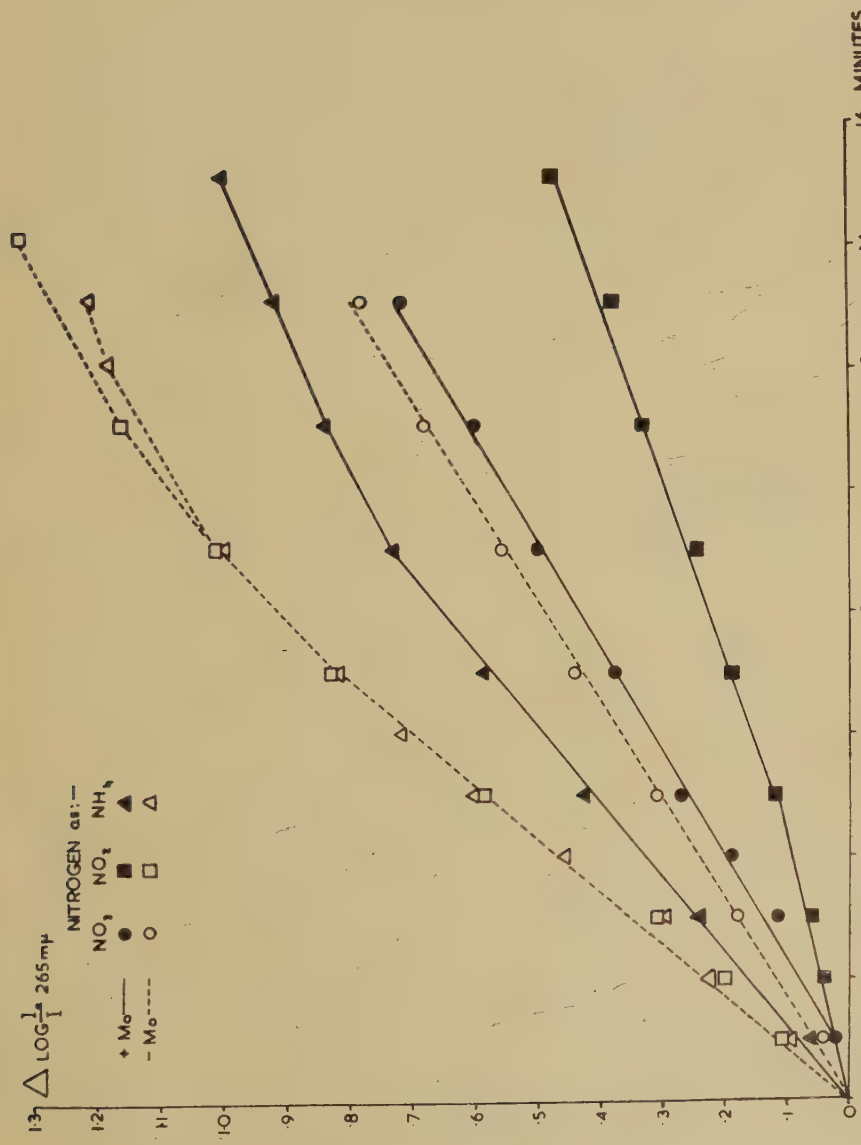


Fig. 1. Rate of destruction of ascorbic acid by soluble enzymes in cell sap expressed from frozen laminae of cauliflower growth with different sources of nitrogen.
——— +Mo; - - - - -Mo.

to five weeks before use. The results from these bulked samples were not consistent, although the activities were apparently as high as or higher than those observed in the previous series. In molybdenum deficient preparations used after the minimum period of storage, the relative activities were usually

greatest in whiptail areas and least in areas from normal leaves. As it was possible that prolonged storage might have affected the activities to different degrees in normal and molybdenum deficient material, this procedure was temporarily suspended.

Cell wall preparations and tissue homogenates :

(1) *Properties.*

Reports by Newcombe (12) and Honda (7) that ascorbic acid oxidase activity is relatively high in, and is possibly associated structurally with, the cell walls of tobacco stem callus and barley roots suggested investigation of similar activity in cauliflower leaf tissues, especially as ascorbic acid oxidation may have effects on pectin degradation in some circumstances (8, 13).

Results illustrated in Fig. 2 confirmed that washed cell wall residues relatively free of finer particles and chlorophyllous material have a high ascorbic acid oxidising activity, and account for the greater part of the total in the tissues as measured in the total homogenate or in the residual supernatant preparations.

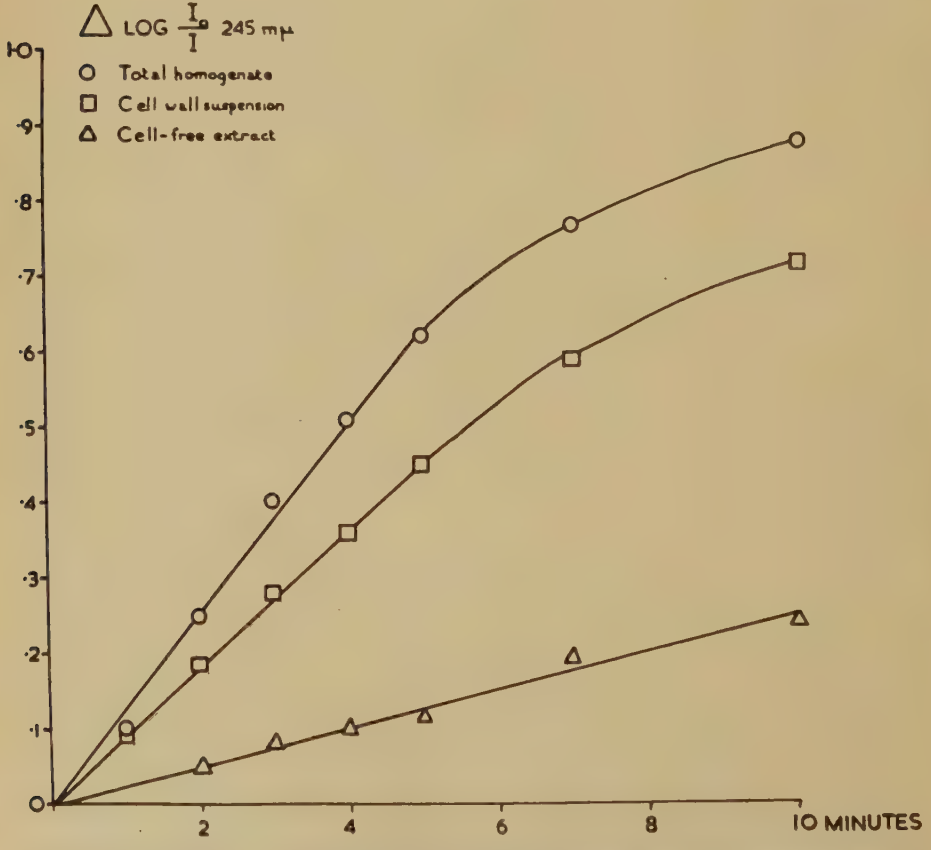


Fig. 2. Distribution of ascorbic acid oxidising activity between cell wall suspensions and cell free preparations of cauliflower leaf.

Results shown in Fig. 3 indicate that the addition of cytochrome c or dihydroxyphenylalanine does not increase the rate of loss of ascorbic acid. Under the conditions used, cytochrome oxidase and tyrosinase activities do not appear, therefore, to contribute to the total activity found in cell wall preparations. There was a slight stimulation of activity by cytochrome c or dihydroxyphenylalanine (DOPA) in total homogenates prepared in 0·4 M sucrose–0·05 M phosphate buffer pH 7·4. 5×10^{-4} M cyanide caused 85–90 per cent. inhibition, but inhibition by 10^{-3} M diethyl dithiocarbamate (DIECA) was only about 35–40 per cent. 5×10^{-2} M ethylenediaminetetraacetic acid (EDTA) also produced about 35–40 per cent. inhibition although this compound is not recorded as an inhibitor of soluble ascorbic acid oxidase preparations (7). The rate of loss of ascorbic acid in the presence of boiled cell wall preparations, or in the absence of added preparations, was less than 5 per cent. of the activity in complete systems.

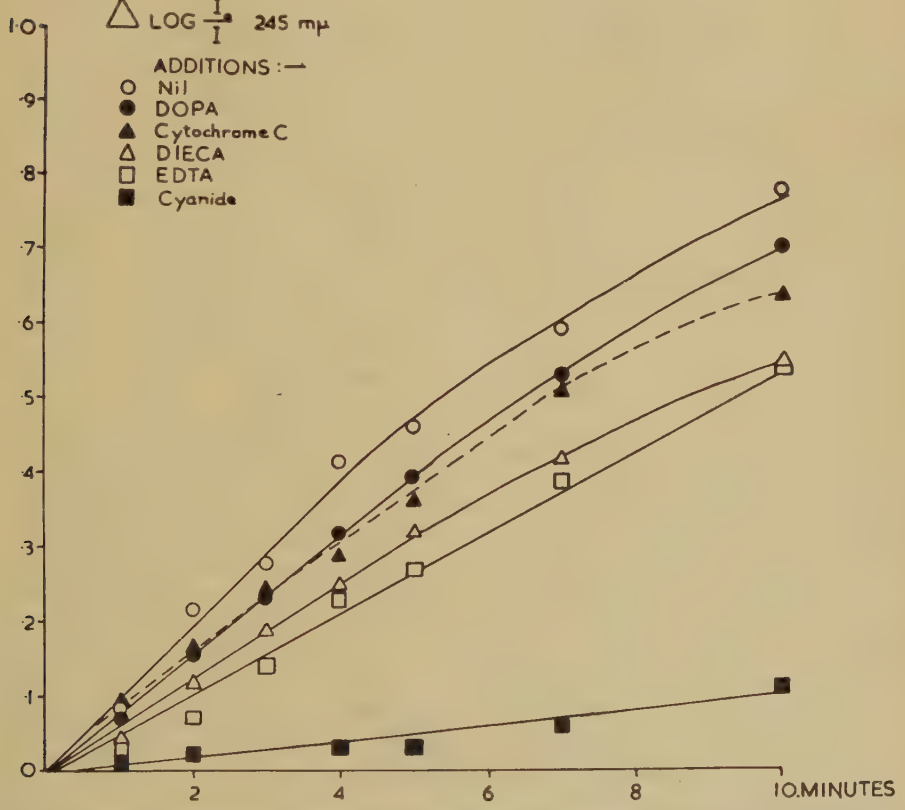


Fig. 3. Effects of some metallo-enzyme inhibitors and of additions of cytochrome c or dihydroxyphenylalanine on activity of ascorbic acid oxidase of cauliflower leaf cell wall suspensions.

The pH optimum for the activity of the cell wall enzyme in 0·1 M phosphate buffer (Fig. 4) was found to be 6·7–7·0, i.e. somewhat higher than that associated with soluble ascorbic acid oxidase preparations or that found for the cell wall suspensions tested by Honda (7).

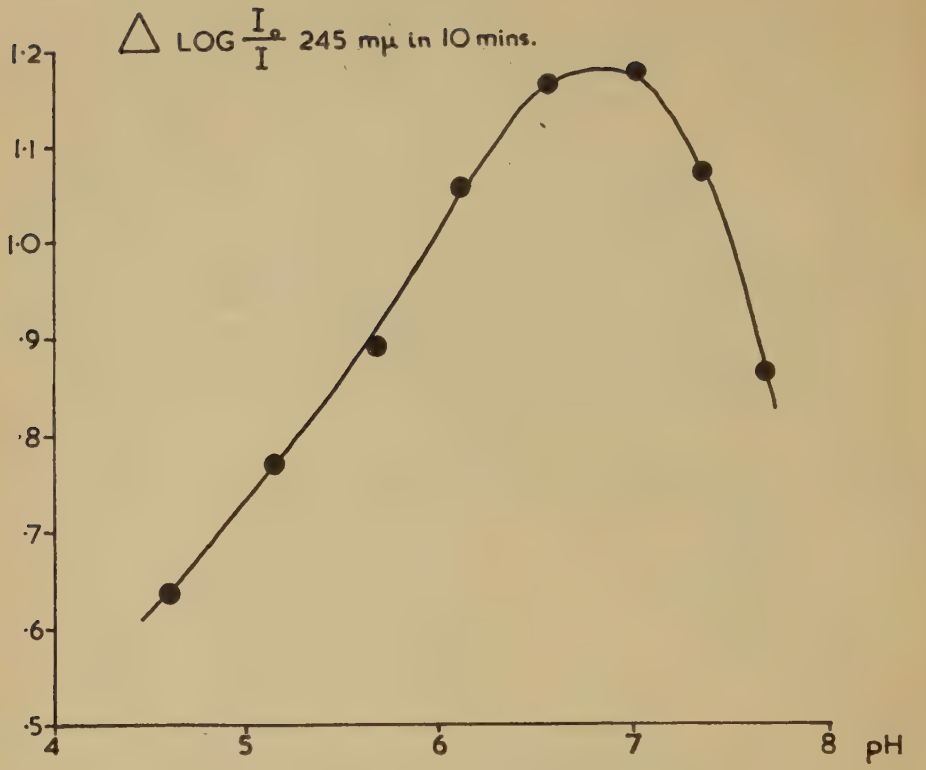


Fig. 4. Relation between pH and activity of cell wall enzyme.

Honda (*loc. cit.*) noted that ascorbic acid oxidase in cell walls did not show the characteristic inactivation during the course of the reaction with the substrate. This point was investigated in the present work. Cell wall suspensions were prepared in bulk and mixed with ascorbic acid and buffer solution pH 6·5 after temperature equilibration. The course of the reaction was followed by sampling at one minute intervals. After 15–20 minutes, part of the reaction mixture was centrifuged ; the cell wall material was re-suspended and washed, and the reaction system was then reconstituted in the original proportions. The remainder of the uncentrifuged mixture was fortified to approximately the original concentration, by additional ascorbic acid contained in a relatively small volume of solution, and the new reaction rate was observed. In some experiments the effects of a second centrifugation and re-suspension, and of a second addition of ascorbic acid, were tested, in such a way that the once-centrifuged and re-suspended fraction was later fortified by ascorbic acid whilst the fraction originally fortified was later centrifuged, washed, re-suspended and tested again.

The results of such an experiment are shown in Fig. 5. There appeared to be initially a progressive partial inactivation of the cell wall enzyme followed by a phase during which activity declined slowly or not at all. The rate of reaction in this possibly linear phase was not increased by raising the ascorbic

acid concentration to the original level. Decrease of substrate concentration was therefore not the cause of this fall in the rate of reaction. On the other hand the suspensions that were centrifuged, washed and re-suspended appeared to regain much of their original activity, which again declined after the initial period and entered the apparently linear phase.

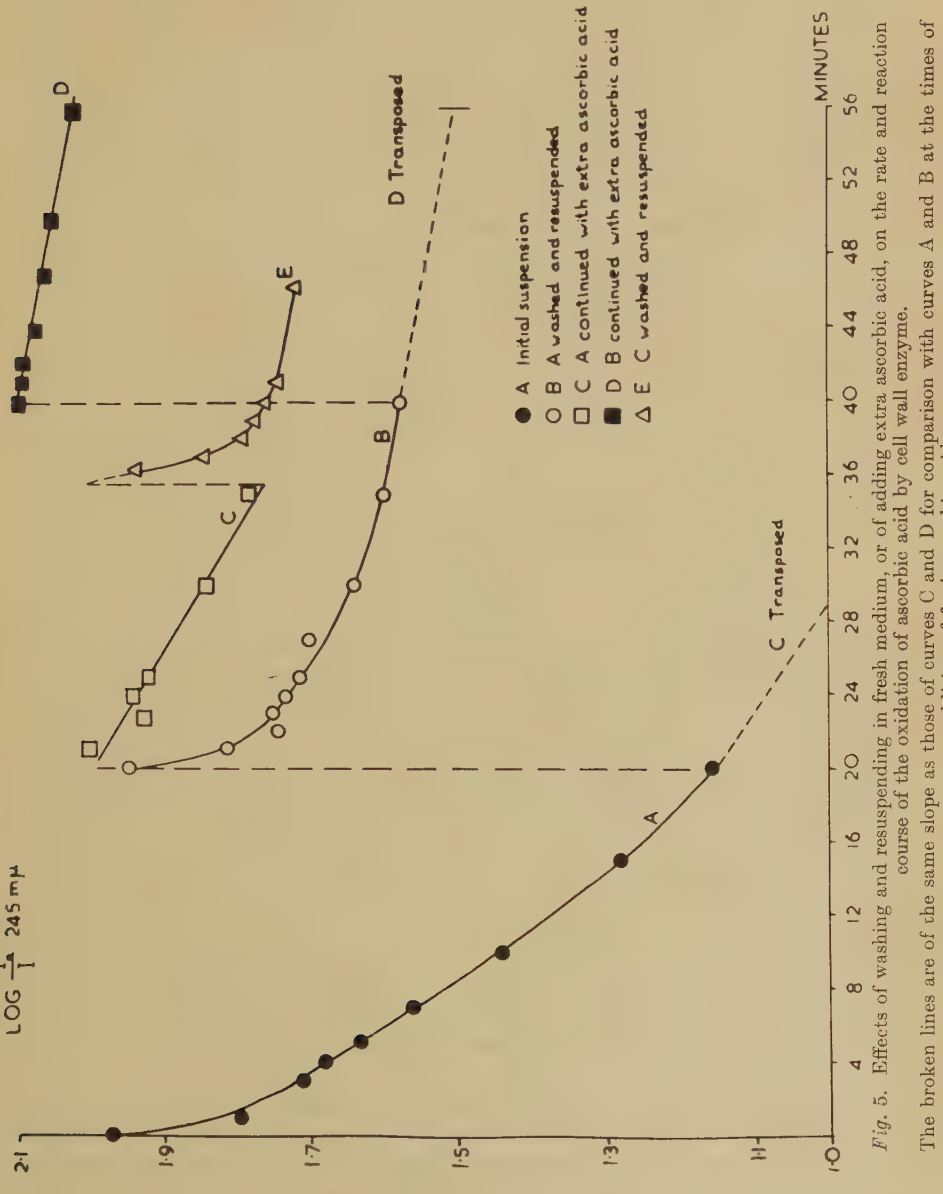


Fig. 5. Effects of washing and resuspending in fresh medium, or of adding extra ascorbic acid, on the rate and reaction course of the oxidation of ascorbic acid by cell wall enzyme.
The broken lines are of the same slope as those of curves C and D for comparison with curves A and B at the times of addition of fresh ascorbic acid.

The transition from the initially rapid but declining rate to the apparently linear rate was not always observed and sometimes it was delayed for several minutes. Under these circumstances the preparations appeared to undergo

little or no inactivation during reaction, as shown in Fig. 2. This type of behaviour was associated with ascorbic acid concentrations around 20 μg. per ml. whilst the changes illustrated in Fig. 5 were associated with concentrations around 100–200 μg. per ml.

The activities of separate replicated preparations, made with 300–400 mg. of tissue on the same day from the same bulk sample of a leaf, were reproducible when assayed over a single time interval of two or three minutes. Preparations made from day to day, however, with apparently comparable material sampled at random from different plants, showed considerable variation in activity. Washed cell wall preparations were relatively stable. They retained full activity for at least 24 hours when stored at 0–4°C. and unbroken leaf tissue retained full activity after storage for 48 hours at —18°C., or for several hours at room temperature. Suspensions that were obtained by centrifuging at 250 × g. were at least as active as those centrifuged at 500 × g. during initial sedimentation. There was no difference between suspensions that were centrifuged for 5 or for 10 minutes. Suspensions washed one to three times with water at 0°C. were as active as unwashed suspensions.

The possibility that varying amounts of ascorbic acid might come into contact with the cell wall enzyme during its extraction before being washed and thus cause varying degrees of initial inactivation was considered. Extractions were made in water; in ascorbic acid solution containing 2 mg./ml., which represented a 5–10 fold excess over that present in the original sample; or in indophenol solution containing 1 mg./ml. which was greatly in excess of the ascorbic acid present in the sample. The activities of these preparations after two washings were almost identical, and there did not appear to be appreciable or lasting initial inactivation by the relatively high concentration of ascorbic acid tested or, therefore, by ascorbic acid originally present in the samples. Grinding in 0·4 M mannitol produced the same activity as grinding in water, although Honda (7) observed enhanced activity of preparations ground in 0·8 M mannitol.

It was concluded from these results that minor variations in the preparation procedure, including the effects of storage for shorter or longer periods at 0° when several preparations were tested during one day, or of different initial ascorbic acid contents of the samples, did not contribute appreciably to the variability between plant treatments and plant replicates.

(2) *Effects of leaf age and plant treatments.*

The activities recorded in Table II for plants of two age groups, as means of 5 or 6 replicates for each batch, were consistently greater in cell wall suspensions from " young " leaves measuring 5–10 cm. in length than in those from " old," i.e. almost mature, though not senescent leaves.

There was no consistent effect of molybdenum supply on the activities of preparations from young or old leaves of plants aged 16 weeks. Preparations from old leaves of plants aged 13 weeks that had been given ammonium sulphate or nitrite without molybdenum showed notably greater activities than those from normal plants in 11 out of 12 consecutive estimations. The source of nitrogen as such had no apparent effect on the activities of preparations from either young or old leaves.

The activities shown by tissue homogenates prepared in 0·4 M sucrose—0·05 M phosphate buffer at pH 7·4 and tested in the presence of 5×10^{-5} M cytochrome c and 3×10^{-4} M dihydroxyphenylalanine bore no consistent relation either to those of the corresponding cell wall suspensions or to the nitrogen and molybdenum treatments in 66 sets of estimations. The activity in homogenates from young leaves was usually greater than that in those from old leaves.

TABLE II.

MEAN RATES OF LOSS OF ASCORBIC ACID, AS μG./MIN./G. FRESH LEAF WEIGHT, IN PRESENCE OF CELL WALL SUSPENSIONS PREPARED FROM LEAVES OF CAULIFLOWER GROWN WITH DIFFERENT NITROGEN AND MOLYBDENUM SUPPLIES.

Source of N	Leaf age	Batch A—16 weeks		Batch B—13 weeks	
		+Mo	—Mo	+Mo	—Mo
NH_4	Old	177	198	138	233
	Young	423	434	498	391
NO_2	Old	263	183	149	346
	Young	419	613	478	485
NO_3	Old	118	114	155	128
	Young	515	317	523	364

Discussion.

The results obtained confirm the observations of Newcombe (12) and Honda (7), that an enzyme resembling ascorbic acid oxidase is localised in or on cell wall residues, and accounts for up to 90 per cent. of the total activity in aqueous tissue suspensions. On the other hand, when cell sap was obtained from cells autolysed by freezing young leaves or from leaves of young plants, the mean activity, calculated on the same basis as used to determine that observed after grinding, corresponded to about 20 per cent. of that shown by suspensions of the cell walls of the young leaves. This activity in cell sap was thus approximately equal to that remaining in the supernatant fraction after separation from the cell wall fraction. It is impossible at this stage to decide whether this correspondence is the result of chance or of the similar partition of the same enzyme systems by the two methods of extraction.

The results of washing and re-suspending cell wall fractions after a period of initial activity suggest that partial inactivation which may occur during reaction may be reversible. This observation to some extent resolves the uncertainty expressed by Honda (7) concerning the identity of the enzyme in barley root cell walls with a true ascorbic oxidase, as he did not observe any inactivation during reaction. The difference between the degree of inactivation observed here and by Honda (7) may be due partly to differences in the methods of measurement of activity which was made over much shorter periods in the work here. Specific differences may also exist between the enzymes in the two types of tissue studied. The possibility that inactivation of soluble ascorbic oxidases is also reversible merits consideration, but the problem is complicated

by the inevitable losses that would be involved in the precipitation required to separate the enzyme from the reaction medium.

Nason *et al.* (11) observed increased polyphenol oxidase activity in molybdenum deficient tomato plants but found no effect of molybdenum supply on soluble ascorbic oxidase. The results obtained hère with cauliflower grown with different nitrogen and molybdenum treatments were not consistent but some points merit comment. Thus cell sap prepared from young plants was more active when they were molybdenum deficient than when they were normal. The difference was particularly marked for plants grown with nitrite or ammonium sulphate where total leaf protein content is not limited by molybdenum deficiency (6). Results obtained with cell wall suspensions from the youngest batch of plants grown with nitrite or ammonium sulphate were relatively consistent and similarly indicated a higher activity in the molybdenum deficient tissues. The great differences observed between the activities in young and old leaves suggest, however, that these studies require to be made in close relation to the developmental stage of the tissues. The preliminary results are nevertheless compatible with the idea that ascorbic acid is more rapidly destroyed *in vitro* by preparations from molybdenum deficient than from normal tissues, and also with the fact that the steady-state concentrations of ascorbic acid are lower in tissues of molybdenum deficient than in those of normal plants (2, 5, 6).

REFERENCES.

(1) Agarwala, S. C. and Hewitt, E. J. (1954). Molybdenum as a plant nutrient : III. *J. hort. Sci.*, **29,** 278.

(2) Agarwala, S. C. and Hewitt, E. J. (1954). Molybdenum as a plant nutrient : IV. *J. hort. Sci.*, **29,** 291.

(3) Agarwala, S. C. and Hewitt, E. J. (1955). Molybdenum as a plant nutrient : VI. *J. hort. Sci.*, **30,** 163.

(4) Hewitt, E. J. (1952). *Sand and water culture methods used in the study of plant nutrition.* Commonwealth Bureau Horticulture, East Malling.

(5) Hewitt, E. J., Agarwala, S. C. and Jones, E. W. (1950). Effect of molybdenum status on the ascorbic acid content of plants. *Nature*, **166,** 1119.

(6) Hewitt, E. J., Agarwala, S. C. and Williams, A. H. (1956). Molybdenum as a plant nutrient : VIII. *J. hort. Sci.* (in the press.)

(7) Honda, S. I. (1955). Ascorbic acid oxidase in barley roots. *Plant Physiol.*, **30,** 174.

(8) Kertesz, Z. I. (1951). *The pectic substances.* Interscience, New York. (1951).

(9) Nason, A. (1952). Metabolism of micronutrient elements in higher plants : II. *J. biol. Chem.*, **198,** 643.

(10) Nason, A., Kaplan, N. O. and Colowick, S. P. (1951). Changes in enzymatic constitution in zinc deficient *Neurospora. J. biol. Chem.*, **202,** 655.

(11) Nason, A., Oldewurtel, H. A. and Propst, L. M. (1952). Role of micronutrient elements in the metabolism of higher plants : I. *Arch. Biochem. and Biophys.*, **38,** 1.

(12) Newcombe, E. H. (1951). Effect of auxin on ascorbic oxidase activity in tobacco pith cells. *Proc. Soc. exp. Biol. N.Y.*, **76,** 504.

(13) Newcombe, E. H. (1955). The use of cultured tissue in a study of the metabolism controlling cell enlargement. *Ann. biol.*, **31,** 195.

PUBLICATIONS, 1955.

(Including those of the A.R.C. Unit of Plant Nutrition)

BEECH, F. W. & CARR, J. C.

(1) Problems involved in the isolation and classification of cider micro-organisms. *Chem. & Ind.*, 1954, 1506–7.

(2) A survey of inhibitory compounds for the separation of yeasts and bacteria in apple juices and ciders. *J. gen. Microbiol.*, **12,** 85–94.
A description of selective media incorporating antibiotics and other organic compounds suitable for the separation of yeasts or bacteria from mixed micro-flora.

BEECH, F. W., CARR, J. G. & CODNER, R. C.

(3) A multipoint inoculator for plating bacteria or yeasts. *J. gen. Microbiol.*, **13,** 408–10.
A description of an inoculator designed for the simultaneous inoculation of plates with a maximum of twenty-four different organisms.

BENNETT, S. H.

(4) The biology, life history and methods of control of the Leaf Curling Plum aphid—*Brachycaudus helichrysi* Kltb. *J. hort. Sci.*, **30,** 252–259.

BOULD, C.

(5) Chelated iron compounds for the correction of lime-induced chlorosis in fruit. *Nature*, **175,** 90–93.
A complete control of lime-induced chlorosis in pear trees was obtained by applying 12 oz. Fe-EDTA or 8 g. Fe-DTPA per tree in April-May. Foliage sprays at 0·1% concentration were also effective with plum and peach.

(6) Lime-induced chlorosis in fruit. *Agric. Review*, **1,** 55–56.

(7) Seasonal changes in the major nutrients of blackcurrant leaves. *J. hort. Sci.*, **30,** 188–196.
Leaf concentrations of N, P, K, Ca and Mg are given and a sampling method is suggested for comparative and diagnostic purposes.

(8) Horticulture. *Rep. Progr. appl. Chem.*, **39,** 666–675.

(9) Bitter Pit in apples. *The Commercial Grower*, December 30, 1955.

BOULD, C., NICHOLAS, D. J. D. & THOMAS, W. D. E.

(10) The effect of washing and a lead acetate soil treatment on the phosphorus content of barley and tomato roots. *Plant and Soil*, **6,** 300–304.
There was no significant difference between the P contents of control roots and those treated with lead acetate prior to washing.

BYRDE, R. J. W. & FIELDING, A. H.

(11) Studies on the acetylesterase of *Sclerotinia laxa*. *Biochem. J.*, **61,** 337–41.
The activity of the enzyme towards phenolic esters of fatty acids decreased with increasing molecular weight of the side chain and was also affected by the phenolic nucleus. The crude extract of *S. laxa* gave four esterase-containing components separable by paper chromatography.

CARR, J. G., PHILLIPS, J. D. (the late), POLLARD, A., WHITING, G. C. & WILLIAMS, A. H.

(12) Quinic acid metabolism in cider fermentation. *Chem. & Ind.*, 1954, 1515–6.
The identification of dihydroshikimic acid as a metabolic product of quinic acid produced by lactic acid bacteria.

CLARKE, G. M.

(13) A design for testing several treatments under controlled environmental conditions. *Applied Statistics*, **4,** 199–206.
A design based on Graeco-Latin squares, used to balance out the most important trends in a laboratory incubator. It has applications to other trials in controlled environments, e.g. fruit storage.

CORKE, A. T. K.

(14) Black currant leaf spot : II. Laboratory tests of fungicides for the prevention of sporing of *Pseudopeziza ribis* on over-wintered leaves. *J. hort. Sci.*, **30,** 197–200.
The successful materials were 0·05% phenyl mercury chloride, 0·2% triethyl tin hydroxide, 0·1% sodium dinitro-*o*-cresylate and 0·1% sodium pentachlorphenate. Cetyl pyridinium bromide was ineffective.

Crang, Alice.

(15) Selecting fruit varieties for canning or freezing. *The Grower*, **43,** 1365–1367.
Most soft fruits can be preserved either by canning or by freezing, but while canning is generally preferred for fruits that need cooking before use, raspberries and strawberries are better frozen. Canning is the better method of preserving all stone fruits with light-coloured flesh, but dark-coloured cherries are usually more attractive when frozen. Dessert pears generally can well, but they are unsuitable for freezing ; culinary varieties of apples can be preserved in either way provided that the sliced or pulped fruit has been boiled before freezing.

(16) The preservation of fruit and vegetables. *Nutrition*, **8,** 118–121.

(17) Freezing your own food. *The Young Farmer*, **22,** 289–291.

(18) Up-to-date methods of food preservation. (Summary of paper given at Gas Sales and Service Conference). *Home Sales and Service*, **21,** 3–4, and *Home Economics*, **1,** No. 10, 13–15.

Greenwood, M.

(19) Sulphur deficiency in groundnuts in Northern Nigeria. *Trans. 5th int. Congr. Soil Sci.*, **3,** 245–251.

Hewitt, E. J. (with Agarwala, S. C.)

(20) Molybdenum as a plant nutrient : III. The interrelationships of molybdenum and nitrate supply in the growth and molybdenum content of cauliflower plants grown in sand culture. *J. hort. Sci.*, **29,** 278.
The molybdenum requirement of the cauliflower is increased by increasing nitrate supply. Whiptail symptoms are produced at approximately 0·00005 p.p.m. Mo in the nutrient solution.

(21) Molybdenum as a plant nutrient : IV. The interrelationships of molybdenum and nitrate supply in chlorophyll and ascorbic acid fractions in cauliflower plants grown in sand culture. *J. hort. Sci.*, **29,** 291.
Ascorbic acid concentration in cauliflower is increased by Mo increments up to 0·0005 p.p.m. Mo, and chlorophyll is increased by increments up to 0·005 p.p.m. Mo.

(22) Molybdenum as a plant nutrient : V. The interrelationships of molybdenum and nitrate supply in the concentration of sugars, nitrate and organic nitrogen in cauliflower plants grown in sand culture. *J. hort. Sci.*, **30,** 151.
Molybdenum deficiency causes a decrease in total and reducing sugars, a decrease in organic nitrogen and an increase in nitrate content in leaves and stems.

(23) Molybdenum as a plant nutrient : VI. Effects of molybdenum supply on the growth and composition of cauliflower plants given different sources of nitrogen supply in sand culture. *J. hort. Sci.*, **30,** 163.
Molybdenum is essential for growth of cauliflower irrespective of the use of a wide range of nitrogen sources. Decreased growth, incidence of whiptail and low ascorbic acid concentration are characteristic of Mo deficient plants.

Hewitt, E. J. (with McCready, C. C.)

(24) Relation of nitrogen supply to the molybdenum requirement of tomato plants grown in sand culture. *Nature*, **174,** 186.
Molybdenum is required by tomato for growth and ascorbic acid content, irrespective of the use of different nitrogen sources, but symptoms of Mo deficiency are associated with plants grown with nitrate.

Hewitt, E. J. (with Miles, Peggy)

(25) The effects of deficiencies and excesses of some mineral nutrients on the growth of tulip and daffodil bulbs in sand culture. *J. hort. Sci.*, **29,** 237.
Tulips are more susceptible to major element deficiencies than daffodils. " Topple " in tulips was caused by calcium deficiency and magnesium deficiency produced marked decrease in growth and characteristic leaf symptoms in tulips.

Hill-Cottingham, D. G.

(26) Photosensitivity of iron chelates. *Nature*, **175,** 347.
The effect of light on dilute solutions of iron chelates is noted and a method for estimating chelated iron, based on the absorption of ultra-violet light, is reported for the first time. It is suggested that photo-reduction may play a part in the release of iron from these complexes after their absorption by plants.

(27) Spectrophotometric determination of iron chelates. *Analyst*, **80,** 906.
The optimum conditions are described for the quantitative estimation of the iron chelates of EDTA, HEEDTA and DTPA.

Luckwill, L. C.

(28) Fruit drop in apples. *R.H.S. Fruit Year Book*, 1955, 76–80.

Luckwill, L. C. & Woodcock, D.

(29) Plant growth regulators : I. The influence of side-chain length on the activity of ω-(2-naphthyloxy)-n-alkylcarboxylic acids for the induction of parthenocarpy in tomatoes. *J. hort. Sci.*, **30,** 109–115.

2-Naphthyloxyacetic acid is highly active but, on lengthening the side-chain, only those compounds having an even number of carbon atoms in the side-chain show activity. All homologues of 3-chloro-2-naphthyloxyacetic acid are inactive.

Marsh, R. W.

(30) Field performance in England of new fungicide sprays for fruit crops. *Proc. 3rd int. Congr. Plant Protection, Paris*, **2,** 614–16.

Results are given of trials of eight organic fungicides against apple scab and mildew, together with records of their phytotoxicity. The eradicant use of phenyl mercury chloride and pentachlorphenol against brown rot and canker is noted.

Marsh, R. W., Martin, J. T. & Crang, Alice.

(31) The control of Botrytis rot (grey mould) of strawberries, and the effects of fungicide spray residues on the processed fruit. *J. hort. Sci.*, **30,** 225–33.

Pre-harvest sprays of 0·25% captan or of 0·4% thiram reduced Botrytis rot on Auchincruive Climax strawberries to approximately one-third of that on the controls : 0·2% tecnazene and 0·2% salicylanilide were ineffective. Stored canned strawberries from the thiram-sprayed plots showed marked tainting ; those from captan-sprayed plots were slightly tainted.

Nicholas, D. J. D.

(32) The application of rapid chemical tests to the diagnosis of mineral deficiencies in horticultural crops : III. *J. hort. Sci.*, **30,** 260.

Potassium, magnesium, calcium, phosphorus and nitrate nitrogen in acetate extracts of potato and cauliflower leaves are better correlated with total amounts of these nutrients in the tissues than are those in citrate, malonate or succinate extracts.

Nicholas, D. J. D. (with Nason, A.)

(33) Role of molybdenum as a constituent of nitrate reductase in soy-bean leaves. *Plant Phys.*, **30,** 135.

(34) Diphosphopyridine nucleotide-nitrate reductase from *Escherischia coli*. *J. Bact.*, **69,** 580.

Molybdenum is shown to be a specific constituent of nitrate reductase, the enzyme that reduces nitrate to nitrite in higher plants and also in bacteria.

Nicholas, D. J. D. & Stevens, H. M.

(35) Valency changes of molybdenum during the enzymatic reduction of nitrate in *Neurospora*. *Nature*, **176,** 1066.

The valency change undergone by molybdenum during nitrate reductase action in *Neurospora crassa* involves Mo^6 and Mo^5 only. Mo^3 will reduce nitrate non-enzymatically but is readily oxidised to Mo^5 in the presence of Mo^6. Mo^4 is very unstable and dismutes readily to Mo^5 and Mo^3 in the presence of water.

(36) Role of molybdenum in oxidation-reduction processes in *Neurospora* and *Azotobacter*. *McCollum-Pratt Symposium on Nitrogen Metabolism : Function of Flavoproteins*. 1955, 178–183.

(37) Role of molybdenum in oxidation-reduction processes in *Neurospora* and *Azotobacter*. *Proc. int. Biochem. Congr., Brussels*, 1955, 29.

Nitrogen fixation and the uptake of molybdenum in *Azotobacter vinelandii* are significantly increased by substituting Mo^5 for Mo^6 in the culture solution.

Pollard, A.

(38) Newer aspects of cider research. *Chem. & Ind.*, 1954, 1506.

(39) Contribution de la Station de Recherches de Long Ashton à l'étiologie du framboisé des cidres. *Ind. Agric. et Alim.*, **72,** 801–2.

A brief account of the Long Ashton work on cider sickness.

Pollard, A., Kieser, Margaret, E. & Timberlake, C. F.

(40) Preparation and storage of soft fruit concentrates. *Food Manufacture*, Sept., 355–8.

A discussion of the effect of storage temperature on quality deterioration in concentrates.

Stringer, A., Woodcock, D. & Skerrett, E. J.
(41) Insecticidal activity and chemical constitution. Analogues and isosteres of DDT. *Ann. appl. Biol.*, **43,** 366–378.
A marked loss of activity against the locust, grain-weevil and cotton stainer follows the replacement of the chlorine atoms of DDT by methyl and hydroxyl groups. The results are reviewed in relation to current theories of DDT structure and activity.

Sturdy, Margaret.
(42) The theory of jam and jelly making. *The Young Farmer,* **23,** 287–291.
(43) Pickles, chutneys and sauces. *Ibid.*, 363–367.

Thomas, W. D. E. & Glynne-Jones, G. D.
(44) The systemic properties of diethyl-S-2-(ethylthioethyl)phosphorothiolate (demeton-S) with reference to the contamination of nectar. *Ann. appl. Biol.*, **43,** 182–191.
Application of ^{32}P-labelled demeton-S to leaves of white mustard, borage and field beans produced no unchanged demeton-S and insignificant amounts of toxic degradation products in the nectar. Appreciable quantities were retained in the leaves.

Thomas, W. D. E., Bennett, S. H. & Lloyd-Jones, C. P.
(45) The absorption, breakdown and systemic behaviour in plants of ^{32}P-labelled demeton-S. *Ann. appl. Biol.*, **43,** 569–593.
Experiments are described involving leaf application to beans, apple and Coleus, and soil application to beans.

Wallace, T.
(46) Fruit research at Long Ashton. *J. R. hort. Soc.*, **30,** 110.

Webb, R. A.
(47) The investigation of mineral deficiencies of some African soils. *Trans. 5th int. Congr. Soil Sci.*, **3,** 214–217.
(48) The mineral deficiencies of some Gambian soils. *Proc. 2nd Inter-African Soils Cong. Leopoldville*, 1954, 419–424.
(49) A new approach to the study of the fertility of tropical soils. *African Soils*, **3,** 379–391.

Williams, A. H.
(50) Paper chromatography of cinnamic acid derivatives. *Chem. & Ind.*, 1955, 120–121.
(51) Phenolic substances of pear-apple hybrids. *Nature*, **175,** 213.

Williams, A. H. (with Cartwright, R. A., Roberts, E. A. H. & Flood, A. E.)
(52) The suspected presence of *p*-coumaryl quinic acids in tea, apple and pear. *Chem. & Ind.*, 1955, 1062–1063.

Woodcock, D.
(53) Fungicidal activity and chemical constitution. Part IV. Synthesis of 5-n-alkyl-8-hydroxyquinoline. *J. chem. Soc.*, 1955, 4391–4393.
Eleven new analogues of 8-hydroxyquinoline have been prepared in an attempt to improve the fungicidal activity.

Woodcock, D. & Byrde, R. J. W.
(54) The metabolism of ω-(2-naphthyloxy)-n-alkyl carboxylic acids by fungi. *Congress Handbook XI Vth int. Congress Pure and Applied Chemistry, Zurich*, 279.
Aspergillus niger, grown in surface culture, is capable of hydroxylating β-naphthyloxyacetic acid and its homologues in the 6-position. β-oxidation also occurs in addition to nuclear hydroxylation.

Woodcock, D. & Pope (Mrs.) P. M.
(55) Synthesis of plant growth-regulators : Part IV. Substituted α-2-naphthyloxypropionic acids. *J. chem. Soc.*, 1955, 577–578.
The preparation of eight new acids and the resolution of α-(3-chloro-2-naphthyloxy) propionic acid using cinchonine are reported in work on the influence of chemical configuration on growth regulating activity.

THE JOURNAL OF HORTICULTURAL SCIENCE

The Journal of Horticultural Science, which is sponsored by the Long Ashton Research Station and the East Malling Research Station, welcomes contributions on horticultural science from Research Institutes at home and abroad. Papers for publication and all communications should be sent to the Editors at East Malling Research Station, Maidstone, Kent.

The current Volume XXXI (1956) contains papers from both the sponsor research stations and, among others, from the John Innes Horticultural Institution, the Scottish Horticultural Research Institute and Reading University. The subjects discussed include studies on the effects of light quality on the growth and development of plants, boron nutrition of the hop, predacious spiders in orchards in south-eastern England, effects of controlled root temperatures on the growth of East Malling rootstocks in water culture, the influence of soil and air temperatures on cropping of glasshouse tomatoes, two methods for the bioassay of auxins in the presence of growth inhibitors, studies on the etiology and mode of spread of Scottish raspberry leaf curl disease, apple mosaic viruses, and cocoa pod production in relation to the incidence of black-pod disease.

The Journal is issued in quarterly parts and is published by Messrs. Headley Bros., 109 Kingsway, London, W.C.2, to whom intending subscribers should apply. The subscription rates are 50 shillings per volume (rate for Canada and the U.S.A. $7.00), or 15 shillings for a single number.